ANALYSIS OF SEXUAL HORMONE BIOSYNTHESIS IN PARASITIC DEVELOPMENT OF *PARASITELLA PARASITICA* ON DIFFERENT HOSTS

A Thesis

Submitted for the Ph.D. Degree in Biology (Microbiology)

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The aim of the present work

This work aimed to determine the range of the trisporic acid system as a signal system within zygomycetes and to find out possible correlations with the host spectrum of *Parasitella parasitica*. Such correlation would contribute in understanding the phylogeny of zygomycetes.

This work was done in five categories as follows:

1. Documentation of the development of parasitic structures of the facultative mycoparasite *Parasitella parasitica* on different hosts.

2. Extraction of sexual hormones in single cultures and in combined cultures of (+) and (-) mating types of zygomycetes as well as in interspecific crossing of the parasite and different hosts.

3. Spectrophotometric analysis of sexual hormones.

4. Thin layer chromatographic analysis of sexual hormones.

5. Bioassay of the activity of sexual hormones using *Mucor mucedo* as a tester strain.

CONTENTS

1. INTRODUCTION

1.1. Sexual hormones

Since the observation of Burgeff (1924), who found that compatible cultures of *Mucor mucedo* produce zygophores when separated by a membrane, the control of sexual reproduction in the heterothallic Mucorales has been thought of in terms of hormones. It was not until 1956, that Burgeff and Plempel obtained clear evidence for the presence of defined chemical substances in the culture medium of mated strains of *M. mucedo*, which induce the production of zygophores in unmated cultures of the same fungus. Plempel elaborated this work in a series of articles (Plempel 1957, 1960, 1963 a, b; Plemple and Braunitzer 1958). He obtained evidence for the presence of two substances in the culture filtrate of mated cultures, one produced by the (-) strain which is active in inducing zygophores in the (+) strain, and the other produced by the (+) strain which is active in inducing zygophores in the (-) strain. He characterized these substances sufficiently to obtain a molecular formula but was not able to separate them, concluding that these hormones are very similar. He also obtained evidence for the presence of two substances, one in each mating type in unmated cultures, that initiated the production of these two hormones in the cultures of the complementary mating types. More recently, van den Ende (1967) extracted a substance active in inducing zygophore production in both mating types of *M. mucedo* from the culture filtrate of mated cultures of *Blakeslea trispora* and found no evidence for the presence of two complementary hormones. Gooday (1968 a, b) extracted and purified the sexual hormone from the mycelium of mated cultures of *M. mucedo*, and he found that the production of the hormone is affected by age and other conditions of the growing cultures.

The signalling substances were named trisporic acids. Trisporic acid substances have been chemically identified from mated cultures of *Mucor mucedo*, *Phycomyces blakesleeanus*, *Blakeslea trispora*, and the homothallic species *Zygorhynchus moelleri* (van den Ende 1968, 1983; Austin *et al.* 1969; Sutter 1970; Sutter *et al.* 1972, 1973, 1974; Sutter and Whitaker 1981 a, b; Sutter and Zawodny 1984).

Trisporic acids are oxygenated, 18 carbon atom-derivatives of ß-carotene (Caglioti *et al.* 1966) and are normally found in the medium of the combined cultures of (+) and (-) mating types of mucoralean fungi. Generally, they are not synthesized in cultures of single mating types.

Cooperative interactions between the complementary mating types is essential for trisporic acid production. The first steps of trisporic acid biosynthesis are thought to be start from ß-carotene to the formation of 4-dihydrotrisporin (Fig. 1), and also are shared by both mating types. The next step is mating type specific. It leads to the formation of 4-dihydromethyl trisporate and methyltrisporate in the (+) mating type and to trisporin and trisporol in the (-) mating type (Sutter *et al.* 1973; Nieuwenhuis and van den Ende 1975; van den Ende 1978; Jones *et al.* 1981; Sutter 1987). In the (+) mating type, trisporic acid can only be synthesized from trisporin or trisporol, and in the (-) mating type only from 4-dihydromethyl trisporate and methyltrisporate (Bu'Lock *et al.* 1973; Sutter *et al.* 1974; Fig. 1). Recently, one of the key enzymes in the biosynthesis pathway, 4-dihydromethyl trisporate dehydrogenase, catalyzing the (-)-specific conversion of 4-dihydromethyl trisporate into methyltrisporate, was isolated and biochemically characterized and its gene has been identified and cloned in *Mucor mucedo* (Czempinski *et al.* 1996). The protein was obtained from the (-) mating types of *M. mucedo* after induction with trisporic acid, and purified by gel filtration and affinity chromatography steps. On SDS-PAGE a band with an apparent molecular mass of 33 kDa was ascribed to the enzyme. After transfering to PVDF membranes, the protein was digested with endoprotease Lys-C and several peptides were sequenced. Oligonucleotides derived from protein sequence data were used for PCR amplification of genomic *M. mucedo* DNA. The PCR fragment was used as probe for isolation of the corresponding cDNA and complete genomic DNA clones. Comparison of protein and DNA sequence data showed that the cloned fragment corresponded to the purified protein. Search for similarity with protein sequences of the Swiss-Prot database revealed a relationship to enzymes belonging to the aldo / keto reductase superfamily. Southern-blot analysis of genomic DNA with the labelled cloned fragment detected a single-copy gene in both mating types of *M. mucedo*. PCR with genomic DNA from other zygomycetes gave rise to several fragments. Hybridization analysis with the cloned *M. mucedo* fragment showed that a fragment of similar length cross-hybridized in *Blakeslea trispora* as well as in *Parasitella parasitica* and *Absidia glauca* (Czempinski *et al.* 1996).

Trisporic acid stimulates carotenogenesis in *Blakeslea trispora* and the development of zygophores (sexual hyphae) in *Mucor mucedo* (Caglioti *et al.* 1966; van den Ende 1968; Austin *et al.* 1970; Bu'Lock *et al.* 1976; White *et al.* 1985; Gooday 1994).

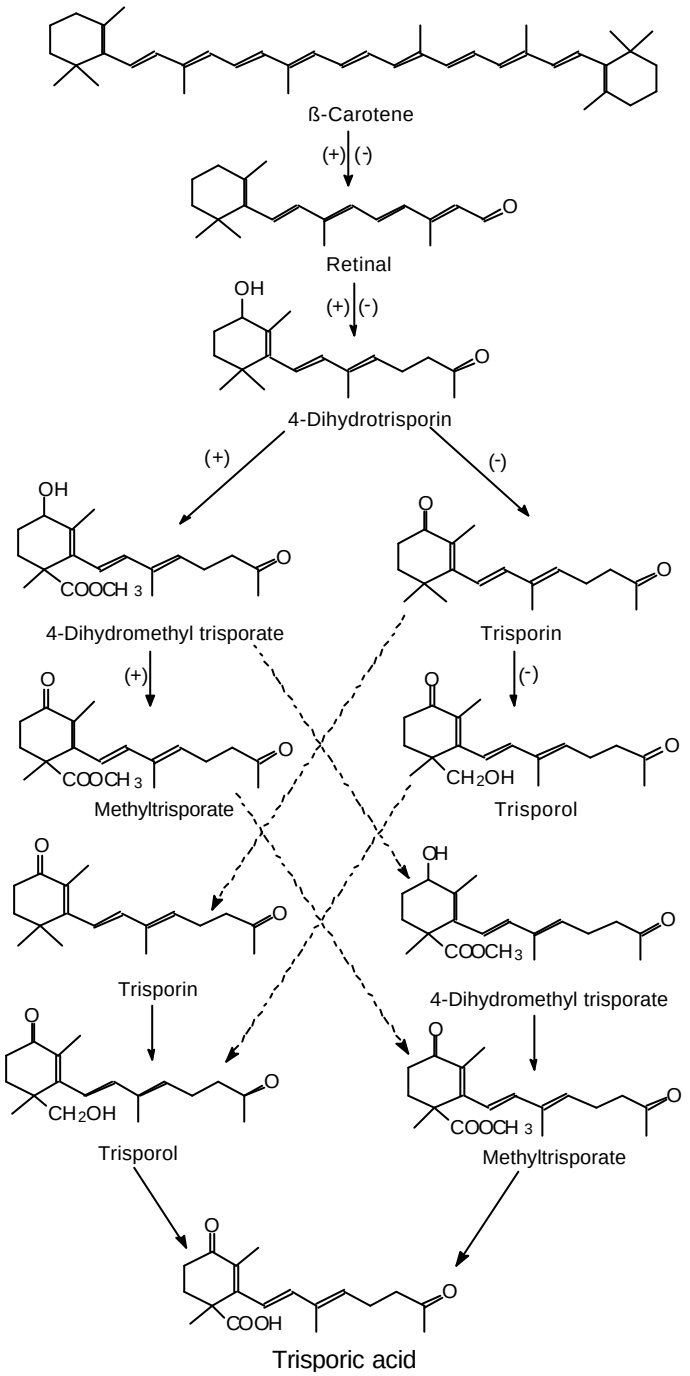


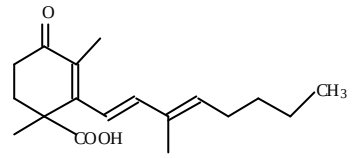
Fig. 1: Collaborative biosynthesis of trisporic acid by cross-feeding of intermediates between (+) and (-) mating types of *Blakeslea trispora*. The dotted lines represent the diffusion of compounds between mating types. The solid lines represent the enzymatic reactions (Sutter *et al*. 1989 and Gooday 1994).

The chemical structures of eight sex-specific pheromones were reported (Bu'Lock *et al.* 1974 b; Nieuwenhuis and van den Ende 1975; Sutter and Whitaker 1981 a). Four sex-specific pheromones were isolated from (+) cultures of *B. trispora* and identified as 4-dihydromethyl trisporates B and C and methyltrisporates B and C. In the (-) cultures, trisporin B and C and trisporol B and C were also isolated and purified. Combined mating type cultures of *B. trispora* accumulate 2 % trisporic acid A, 15 % trisporic acid B, and 83 % trisporic acid C (Caglioti *et al.* 1966). In contrast, trisporic acid E represents 30 % of the total trisporic acids made by combined mating type cultures of *Phycomyces blakesleeanus*, while trisporic acid C was 40 % (Miller and Sutter 1984, Fig. 2). Sutter *et al.* (1974) stated that 35 % of the trisporic acids made by (+) cultures of *B. trispora* incubated with extracts from (-) cultures migrated slower on silica gel than trisporic acid C, supporting the possibility that *B. trispora* single cultures might produce trisporic acid E. More recently, Sutter *et al.* (1989) found three trisporic acids in cultures of *B. trispora* in which (+) and (-) mating types were separated by a membrane. Two of the trisporic acids were new compounds, trisporic acid D and trisporic acid E. The structure of the third was previously described by Miller and Sutter (1984) as methyl trisporate-E with a hydroxyl group at C-2. Trisporic acid D and Trisporic acid E accounted 9 % of the total trisporic acids produced by *B. trispora* (Fig. 2).

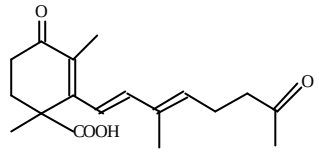
1.2. Sexual reactions

Sexuality in the Mucorales is based on the interaction of (+) and (-) mating types in heterothallic species, and on the interaction of (+) and (-) parts of the same mycelia in the homothallic species. Sex-specific substances are secreted by both (+) and (-) mating types, resulting in the production of sex hormones, trisporic acids, which initiate the formation of zygophores and probably also the mutual attraction prior to wall fusion and production of progametangia and gametangia. These first stages in the sexual process indicate that (+) and (-) strains interchange signals and that the reaction is mating type-specific (Blakeslee 1904; Stalpers and Schipper 1980).

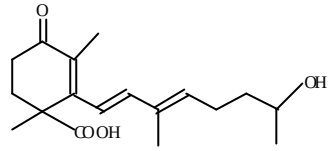
Sexual reaction can be induced by (+) and (-) strains of different species. The fusion of (+) and (-) gametangia, the first step towards zygospore formation, is found only in matings of



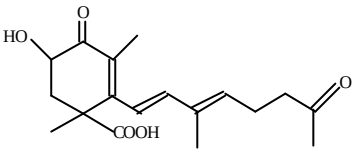
Trisporic acid A



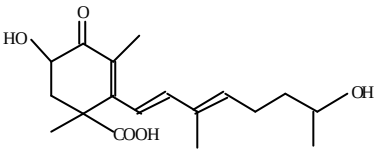
Trisporic acid B



Trisporic acid C



Trisporic acid D



Trisporic acid E

Fig. 2: Chemical structures of trisporic acids as reported by Bu'Lock and Winstanley (1971) and Sutter *et al.* (1989).

compatible strains of the same species. Both partners are involved in the formation of zygospores. After gametangial fusion a new zygospore wall is formed against the gametangial wall, subsequently breaking through at various places to form wart-like structure. There is much variation in the final appearance of mature zygospores of *Mucor* and related genera, due to the fact that the development may stop at any stage. Moreover there is considerable variation in the degree of splitting, the length, and the length-width ratio of the arms. The sequence of events of sexual process was observed in *Mucor mucedo*. When plus and minus strains of this fungus growed together on a solid substrate, erect progametangia are formed in both mycelia without any detectable physical contact occurring between the two sexual partners. Progametangia formation is obviously induced by diffusing of one mating type to the other. The progametangia grow into contact with another by a process of mutual attraction, followed by the formation of gametangia. Finally fuse and give rise to zygospores. Progametangia of *M. mucedo* being well distinguished from vegetative hyphae. Consequently, most of the work has been done with this species. There is ample evidence that many other species of order Mucorales are similar to *M. mucedo* in respect to their sexual mechanism. Incomplete matings have frequently been observed between different species, sometimes even between species beloging to different genera (Burgeff 1924; Blakeslee and Cartledge 1927; Blakeslee *et al.* 1927; Plempel 1963 b; van den Ende and Stegwee 1971). On the other hand, failure to fuse is caused not only by interspecific incompatibility, but also by physiological and environmental conditions. Partners isolated from the same location are often more prolific than partners isolated from different locations (Stalpers and Schipper 1980).

Occasionally, a single gametangium gives rise to an azygospore. Two gametangia may be present, but in the absence of lysis of the fusion wall, an azygospore may develop from one of the gametangia. Azygospores are known in both hetero- and homothallic species, usually arising spontaneouslly in association with matings, rarely following induction by specific distinct partners. Some heterothallic species have a pronounced tendency towards azygospore production in intraspecific matings, e.g. *Mucor indicus* and *M. hiemalis* (Stalpers and Schipper 1980). In this respect, Ling Young (1930) obtained an increased number of azygospores in intraspecific matings by growing one partner on a favourable medium and the other on an unfavourable one. Cutter

(1942) studied a strain of *Zygorhynchus moelleri*, which very infrequently produced zygospores, the majority of the zygospore-like structures being azygospores.

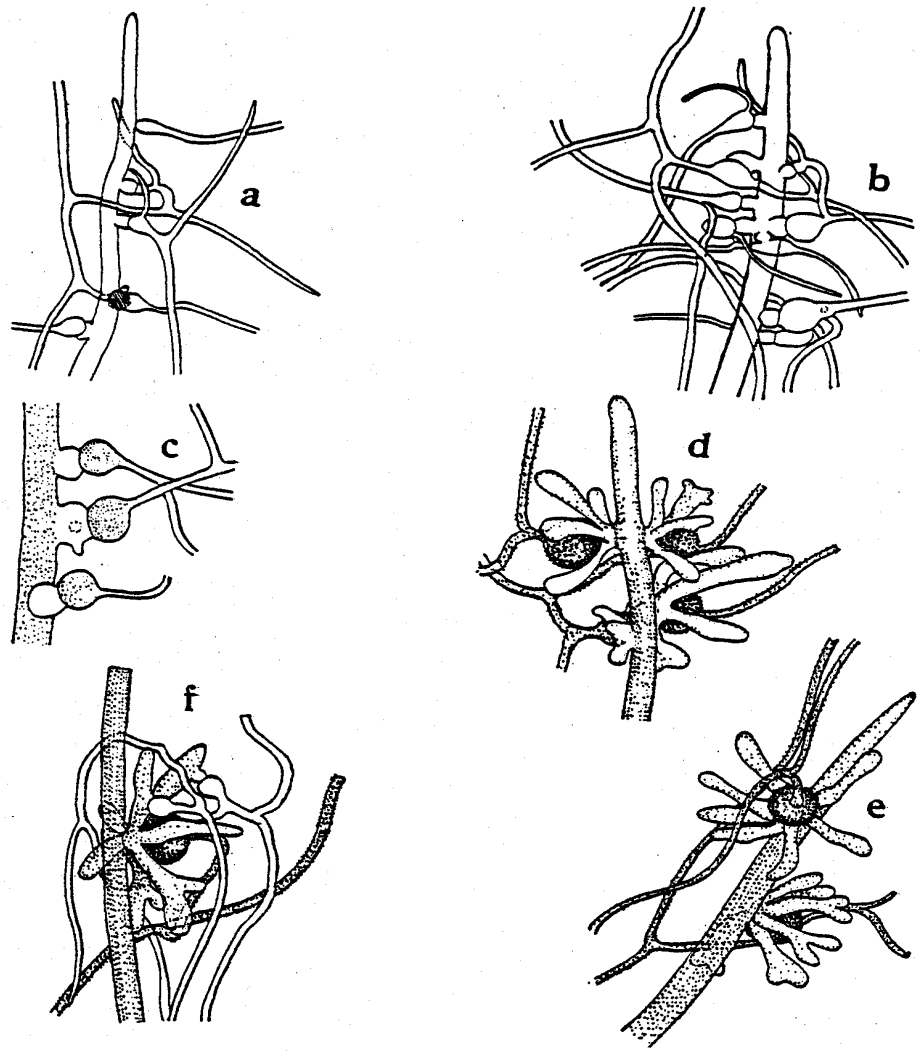
Sexuality in Mucorales is often accompanied by a considerable increase in carotene synthesis. *B. trispora* is known for a substantial increase in extractable carotenoids in mated cultures compared with unmated cultures (Anderson *et al.* 1958; Hesseltine 1961). The formation of progametangia inducers is accompanied by stimulation of carotene synthesis in *B. trispora*, but the stimulation is not observed when cultures of single strains are treated with purified samples of these substances (Plempel 1965). Their biosynthesis depends on the biosynthesis of carotenoids.

Zygospore formation in the Mucorales was known to be sporadic and unpredictable. Some isolates readily formed zygospores, but others never formed zygospores. This confused situation was clarified in the classic paper by Blakeslee (1904) in which he described the two forms of mating systems in these fungi. A minority of species were homothallic, mating between adjacent hyphae of the same mycelium, while the majority of species were heterothallic with zygospore formation occurring only between individuals of two mating types of the same species, designated (+) and (-). He also described the phenomenon of interspecific sexual reactions in which complementary mating types of different species interact, sexual differentiation occure where the two cultures meet. These interactions allow the mating type (+), (-) or neutral of all isolates of Mucorales to be determined by mating them with strains of *Rhizopus nigricans* used in Blakeslee's original work. In an extensive survey using many neutral isolates of 36 species, Blakeslee *et al.* (1927) showed that both mating types were widely distributed (393 (+) and 470 (-)) but that 235 isolates were neutral. Interspecific reactions were also seen between two homothallic species, or between homothallic and heterothallic species. Some homothallic species seem to have more pronounced (+) or (-)-specific behaviour. *Zygorhynchus moelleri* only conjugated with heterothallic plus species, while *Z. heterogamus* has the opposite tendency (Satina and Blakeslee 1930). The intensities of the interspecific reactions vary, but usually they are confined to zygophore formation with or without zygotropism and progametangium formation (Blakeslee *et al.* 1927).

1.3. Parasitic interactions

When the German botanist Hans Burgeff (1924) described the sexual and parasitic interactions in the *Mucor*-like fungus *Parasitella parasitica* (synonyms: *P. simplex* and *Mucor parasiticus*) at the microscopic level, he reported among many important observations that the chemical principle inducing sexual development in these fungi seemed to be universal. A (+) mating type of a given species will induce early sexual reactions, i.e. zygophore formation, in many different species only if they belong to the (-) mating type. Burgeff also described the interactions between aerial mycelia of the facultative mycoparasite *P. parasitica* and some of its hosts, which are also members of zygomycetes. The most important observation was that the cell walls of both partners are dissolved in the contact zone. Cytoplasm and cell organelles from the parasites, including nuclei, invade the host's mycelium. Recently this kind of mycoparasites have been classified as fusion biotrophs (Joffries and Young 1994). In some instances the fusion zones between host and parasite are quite small, and certainly not suitable for the transfer of organelles. In the parasitic interaction, however, the resulting cytoplasmic continuum leads to the transfer of genetic material from the parasite to its host (Burgeff 1924; Kellner *et al.* 1993; Wöstemeyer *et al.* 1995). At the end of a complex differentiation program, the host develops a gall-like structure adjacent to the infection site, whereas the parasite forms persistent sikyospores (Fig. 3). In this respect, Satina and Blakeslee (1926) had investigated the parasitic interaction of *Parasitella simplex* with 54 isolates belonging to 9 genera and 17 species of mucoralean fungi. Both homothallic and heterothallic forms were selected as hosts and showed different levels of parasitic strength for the various species tested.

Parasitella parasitica is a heterothallic facultative mycoparasite which belongs phylogenetically to the *Mucor* group within the Zygomycetes (Voigt and Wöstemeyer 2001). It is a facultative parasite of several, but not all, mucoralean fungi. Recently, the parasitic interaction and transfer of genetic material from the parasite into the host in the host-parasite system *P. parasitica-A. glauca* have been extensively studied. The interactions between host and parasite are found to be dependent on several factors. One of these factors being that infection of some species of Mucorales, like the host organism *Absidia glauca*, were found to be strongly mating type-specific. (+) strains of *P. parasitica* infect exclusively (-) strains of *A. glauca*, whereas (-)



[From: Burgeff, 1924]

Fig. 3: Infection pathway of the mycoparasite *Parasitella parasitica* and its host. Developing stages, (a) contact between specialized parasitic hyphae of *P. parasitica* and its host. (b) Swelling of the parasite's hyphal tip, septum formation in the parasite. (c) Fusion of the primary sikyotic cell with the host mycelium, invasion of the *Parasitella* nuclei into the host mycelium and swelling of the parasite's region adjacent to the primary sikyotic cell to a bulb-like structure. (d) The formation of secondary sikyotic cell, formation of side branches. (e, f) Side branches develop into a gall-like structure, differentiation of the secondary sikyotic cell into a persistent sikyospore.

strains of the parasite are required for the infection of (+) type hosts (Wöstemeyer *et al.* 1995, 1997 a). Also the host-parasite recognition is supposed to be mediated by the same trisporic acid system as it is required for communication between sexual partners within Mucorales. Measurements of trisporic acid levels in cocultures of *P. parasitica* with *A. glauca* revealed, that the interaction with a host organism stimulates the cooperative biosynthesis of trisporic acid (Wöstemeyer *et al.* 1997 a, b).

Physiology of sex in Mucorales as well as in the other orders of zygomycetes have important considerations to research. We must have more evidence for the ability of different homothallic and heterothallic species of zygomycetes to produce trisporoids in their culture medium. The production of trisporoids was detected in members of Mucorales, e.g. *Blakeslea trispora* and *Mucor mucedo*. In this work, trisporoids are found to be produced in the culture medium of several homothallic and heterothallic species of Mucorales as well as in other orders of zygomycetes, including Mortierellales and Kickxelalles. Trisporic acid is well knoun for the regulation of sexual reactions in Mucorales and also possibly to regulate such reaction in Mortierellales and Kickxelalles. In this work, the relationships between the concentration of trisporoids in the culture medium of zygomycetes and the strength of sexual and parasitic interactions was also discussed. One of the most important observations in this respect, that the transfer of genetic material from the parasite into its host through the parasitic process. The extent of parasitic interaction development between the mycoparasite *P. parasitica* and different host species are detected in this study. These hosts are members of homothallic and heterothallic zygomycetes. This work not only restricted to the ability of *P. parasitica* to infect several members of mucoralean fungi but also aimed to study such reactions with other members of zygomycetes such as Mortierellales and Kickxellales. Trisporoids were extracted and analysed in both single cultures and in intraspecific interactions of all zygomycetes as well as in the cultures of interspecific interactions of *P. parasitica* and different hosts to have more evidence for the correlation between the production of trisporoids and the extent of parasitic development.

2. MATERIALS AND METHODS

2.1. Organisms and cultivation conditions

2.1.1. Strains studied

The strains of fungi listed in Table 1 were used in this study and are available from the Fungal Reference Center, Friedrich Schiller University, Jena, Germany. The classification of the zygomycetes studied in this work are found in Table 2.

2.1.2. Cultivation

For inoculum production all zygomycetes were grown on solid medium containing 30 g malt extract and 15 g agar per litre.

2.1.3. Extraction of trisporoids

2.1.3.1. Preculture medium

All strains were precultured in 500 ml flasks containing 100 ml of modified supplemented minimal liquid medium (Wöstemeyer 1985) with the following chemical composition: 10 g maltose, 1 g NH_4Cl, 4 g KH_2PO_4, 0.9 g K_2HPO_4, 0.25 g $MgSO_4$. $7H_2O$, and 5 g yeast extract per litre. Each flask was inoculated with three 1 cm squares of mycelium cut from a culture grown on solid medium.

2.1.3.2. Maltose solution

The combined cultures were grown in 500 ml flasks with 100 ml maltose solution, containing 20 g maltose and 0.1 g $(NH_4)_2HPO_4$ per litre.

2.1.4. Biological activity of trisporoids

All fungal extracts were bioassayed on the zygophore induction medium containing 20 g maltose, 10 g KNO_3, 5 g KH_2PO_4, 2.5 g $MgSO_4$. $7H_2O$, 1 g yeast extract and 12 g agar per litre (van den Ende 1968, modified)

2.1.5. Parasitic and sexual interactions

All strains were grown on solidified supplemented minimal medium in 10 cm Petri dishes. The chemical composition of this medium was described before for the extraction of trisporoids.

Table 1: Strains used in this work

Strains	Source	Origin
Absidia glauca (+)	FSU-329	CBS 100.48
Absidia glauca (-)	FSU-330	CBS 101.48
Absidia glauca (+)	FSU-659	ATCC 6776a
Absidia glauca (-)	FSU-660	ATCC 6776b
Absidia orchidis	FSU-323	P 2
Absidia parricida	FSU-547	CBS 174.67
Absidia spinosa	FSU-550	'Tina 1' Greifswald
Actinomucor elegans	FSU-276	P 201
Blakeslea trispora (+)	FSU-331	CBS 130.49
Blakeslea trispora (-)	FSU-332	CBS 131.49
Chaetocladium brefeldii	FSU-284	P 164
Coemansia formosensis	FSU-782	DSM 6934
Cunninghamella elegans	FSU-270	P 93
Gilbertella persicaria	FSU-326	P 181
Gongronella butleri	FSU-266	P 105
Halteromyces radiatus	FSU-299	P 266
Linderina macrospora	FSU-700	CBS 375.66
Mortierella gamsii (+)	FSU-824	CBS 551.73
Mortierella gamsii (-)	FSU-825	CBS 552.73
Mortierella globulifera (+)	FSU-827	CBS 154.76
Mortierella globulifera (-)	FSU-826	CBS 857.70
Mortierella humilis (+)	FSU-828	CBS 443.68
Mortierella humilis (-)	FSU-829	CBS 745.68
Mortierella indohii (+)	FSU-831	CBS 460.75
Mortierella indohii (-)	FSU-830	CBS 331.74
Mortierella minutissima (+)	FSU-832	CBS 277.71
Mortierella minutissima (-)	FSU-833	CBS 278.71
Mortierella parvispora (+)	FSU-834	CBS 315.61

Table 1: Continued

Strain	Source	Origin
Mortierella parvispora (-)	FSU-835	CBS 316.61
Mucor mucedo (+)	FSU-621	CBS 144.24
Mucor mucedo (-)	FSU-620	CBS 109.16
Mucor racemosus (+)	FSU-624	CBS 225.37
Mucor racemosus (-)	FSU-625	CBS 226.37
Mycotypha africana	FSU-296	P 159
Parasitella parasitica (+)	FSU-387	CBS 412.66
Parasitella parasitica (-)	FSU-388	ATCC 11077
Phycomyces blakesleeanus (+)	FSU-2486	A 56
Phycomyces blakesleeanus (-)	FSU-2487	NRRL 1555
Pilaira anomala	FSU-268	P 171
Syncephalastrum racemosum	FSU-290	P 130
Syzigites megalocarpus	FSU-728	P 182
Thamnidium elegans (+)	FSU-702	CBS 342.55
Thamnidium elegans (-)	FSU-701	CBS 341.55
Thamnostylum piriforme	FSU-278	P 101
Zygorhynchus moelleri	FSU-531	P 67

Explanation of abbreviations:

FSU = Friedrich Schiller University, Jena, Germany.

CBS = Centraalbureau voor Schimmelcultures, Netherlands.

ATCC = American Type Culture Collection.

P = Phycomycetes, Fungal Reference Center, Jena, Germany.

DSM = Deutsche Stammsammlung von Mikroorganismen, Germany.

NRRL = Norther Regional Research Laboratories, America.

A 56 = Originated from Salamanca, Spain, A.P. Eslara, M.I. Alvarez.

Table 2: Classification of zygomycetes

Systematic criteria	Genera and species	Type
O: Kickxellales		
F: Kickxellalaceae	*Coemansia formosensis*	homothallic
	Linderina macrospora	„
O: Mortierellales		
F: Mortierellaceae	*Mortierella gamsii*	heterothallic
	M. globulifera	„
	M. humilis	„
	M. indohii	„
	M. minutissima	„
	M. parvispora	„
O: Mucorales		
F: Absidiaceae	*Absidia glauca*	heterothallic
	A. orchidis	homothallic
	A. parricida	„
	A. spinosa	„
F: Chaetocladiaceae	*Chaetocladium brefeldii*	homothallic
F: Choanephoraceae	*Blakeslea trispora*	heterothallic
F: Cunninghamellaceae	*Cunninghamella elegans*	homothallic
F: Gilbertellaceae	*Gilbertella persicaria*	homothallic
F: Mucoraceae	*Actinomucor elegans*	homothallic
	Gongronella butleri	„
	Halteromyces radiatus	„
	Mucor mucedo	heterothallic
	M. racemosus	„
	Parasitella parasitica	„
	Syzigites megalocarpus	homothallic
	Zygorhynchus moelleri	„
F: Mycotyphaceae	*Mycotypha africana*	homothallic
F: Phycomycetaceae	*Phycomyces blakesleeanus*	heterothallic
F: Pilobolaceae	*Pilaira anomala*	homothallic
F: Syncephalastraceae	*Syncephalastrum racemosum*	homothallic
F: Thamnidiaceae	*Thamnidium elegans*	heterothallic
	Thamnostylum piriforme	homothallic

O = Order F = Family

2.2. Experimental techniques used in this work

2.2.1. Extraction of trisporoids

Trisporoid substances were extracted in two successive steps at pH 2 and pH 8, to favour the separation of the acid and the alkaline to neutral trisporoids, modified according to the procedure described by van den Ende *et al.* (1972) and Sutter and Whitaker (1981a). All strains (1 litre total culture volume each) were precultured in supplemented minimal liquid medium for 5-6 days in the natural day light/dark regime at 20°C with continuous agitation. The flasks were harvested by filtration, the combined mycelia were rinsed with maltose solution to remove residues of supplemented minimal medium, and transferred into other flasks containing 100 ml maltose solution each. Flasks were incubated on a rotary shaker in the dark for 5-7 days. At the end of the incubation period, flasks were filtered and the filtrates were used for the extraction of trisporoids. The pH of the filtrates was adjusted to pH 8 by 1M KOH solution. The filtrates was mixed with half a volume of trichloromethane and 2-propanol solvent (100 plus 5 v/v). The mixture was shaken well and after separation of the phases, the chloroforme phase was collected in brown glass bottles and dried over Na_2SO_4 to remove residual water. The bottles kept in the dark overnight. The residual culture filtrates were adjusted to pH 2 by adding 10 % HCl solution, mixed with the same amount of the solvent and extracted again. The pH 2 extract was treated in the same way as the pH 8 extract. The Na_2SO_4 was removed by filtration. Trichloromethane was removed by evaporation in a rotary vacuum concentrator. The residue was dissolved in 100 % Ethanol (1.5 ml for each sample) and stored in small brown glass bottles at -20°C until further use.

2.2.2. Spectrophotometric analysis

Aliquots of the extracts were taken for spectrophotometric analysis. Their absorbance spectra were recorded and the absorbance values were measured at 285 and 325 nm. (V-560 UV/VIS spectrophotometer, JASCO, Tokyo, Japan). Trisporoid concentrations were calculated using the specific extinction coefficients for trisporic acid, $E^{1\%\,cm}_{325} = 572$, and for trisporic acid precursors, $E^{1\%\,cm}_{285} = 547$, (Nieuwenhuis and van den Ende 1975).

2.2.3. Thin layer chromatography

2.2.3.1. Analysis of culture extracts

Aliquots of the extracts containing 20 µg of trisporoids were taken and subjected to thin layer chromatography (tlc) on silica gel, (20X20 cm, 0.25 mm thick., F254, Merck, Darmstadt). The plates were developed in a solvent mixture consisting of 50 % trichloromethane, 30 % acetic acid ethyl ester, 15 % tertiary butyl methyl ether and 5 % glacial acetic acid. After 1 hour the developed plates were air dried and photographed on a UV-transilluminator with an excitation wavelength of 312 nm.

2.2.3.2. Molecular analysis of single bands developed on the thin layer chromatography

Aliquots containing 400 µg of trisporoids were subjected to preparative tlc. The darkest bands were carefully marked and then removed individually from the tlc plates by scraping off the silica gel. The silica gel fractions were eluted into 1 ml of 100 % ethanol each in Eppendorf tubes. The tubes were centrifuged at 6000 r.p.m for 5 minutes to sediment the silica gel. The clear supernatant was centrifuged again for 5 minutes to remove further traces of silica gel. Absorbance spectra of the tlc fractions were recorded to determine the molecular type of trisporoids.

2.2.4. Biological activity of trisporoids

Aliquots containing 10 µg of trisporoids were applied to filter discs of 4 mm diameter. These discs were allowed to dry and placed on the agar surface in front of 4-5 day-old growing cultures of (+) and (-) strains of *Mucor mucedo*, respectively. The dishes (4 dishes for each sample) were incubated in the dark at 20°C. After one day, the dishes were examined by dissecting microscope and the number of zygophores was counted. The average number of zygophores in the four replicates was calculated (van den Ende and Stegwee 1971; Werkman and van den Ende 1973; Sutter and Whitaker 1981b).

2.2.5. Parasitic and sexual development of *P. parasitica* and different zygomycetes

Parasitic and sexual interactions were studied on the supplemented minimal agar medium. To investigate the ability of some strains of zygomycetes to interact parasitically with *P. parasitica*, these strains were cultivated together with *P. parasitica* (+) or (-) strains. The dishes

were incubated in the dark at 20°C. From the third day of inoculation the dishes were extensively examined for parasitic development and sikyospore formation using a dissecting microscope. Photographs were taken for positively and negatively interacting strains. Sexual development and zygospore formation between (+) and (-) mating types of each heterothallic species were examined by the same method (Burgeff 1924; Satina and Blakeslee 1926).

Table 3: Chemicals used in this work and their sources

Chemicals	Sources
Acetic acid ethyl ester	Carl Roth GmbH + Co, Karls ruhe, Germany.
Agar	Otto Nordwald KG, Hamburg, Germany.
Amonium chloride	VEB Jenapharm, Laborchemie, Apolda, Germany.
Di-amonium hydrogen phosphate	Merck, Darmstadt, Germany.
Di-potasium hydrogen phosphate	Merck, Darmstadt, Germany.
Ethanol	Bundesmonopolverwaltung für Branntwein, Offenbach, Germany.
Glacial acetic acid	Carl Roth GmbH + Co, Karlsruhe, Germany.
Hydrochloric acid	Merck, Darmstadt, Germany.
Magnesium sulphate	VEB Jenapharm, Laborchemie, Apolda, Germany.
Malt extract	Biomalz-GMBH, Teltow, Germany.
Maltose	Merck, Darmstadt, Germany.
Potasium di-hydrogen phosphate	Merck, Darmstadt, Germany.
Potasium hydroxide	VEB Chemiekombinat Bitterfeld, Germany.
Potasium nitrate	Merck, Darmstadt, Germany.
Sodium sulphate	Merck, Darmstadt, Germany.
Tertiary butyl methyl ether	Merck, Darmstadt, Germany.
Trichloromethane	Carl Roth GmbH + Co, Karlsruhe, Germany.
2-propanol	Merck, Darmstadt, Germany.
Yeast extract	Carl Roth GmbH + Co, Karlsruhe, Germany.

3. RESULTS

3.1. Analysis of culture extracts

Trisporoids are known to regulate the sexual and parasitic reactions in members of homothallic and heterothallic mucoraceous fungi including *Blakeslea, Mucor*, *Phycomyces*, *Parasitella*, *Absidia* and *Zygorhynchus.*

Thirty species of zygomycetes were analysed for their ability to produce trisporoids in both single cultures and in interspecific combinations with *Parasitella parasitica*. The UV-absorbance values were measured within the specific region of trisporoid absorbance at 285 and 325 nm.

The data obtained from the analysis of culture extracts in table (5) reveal that all *Absidia glauca* strains tested have the ability to produce UV-absorbing substances including trisporoid compounds. The amount of such substances was varied among the different *A. glauca* strains. *A. glauca* $_{6776}$ (-) produced the highest amount of UV-absorbing substances. The UV-absorbance spectra of single culture extracts as well as the combination extracts did not give any evidence for the presence of trisporoids in high concentrations.

Analysis of the combined culture extracts of *A. glauca* and the mycoparasite *P. parasitica* reveal different levels of UV-absorbing substances in the different interspecific combinations. The interspecific interaction of *P. parasitica* with *A. glauca*$_{100.48}$ (+) or *A. glauca*$_{101.48}$ (-), respectively, stimulated the biosynthesis of UV-absorbing substances including trisporoids, expressed in higher absorbance values measured in pH 2 extracts. The pH 8 combined culture extracts, on the other hand showed different levels of stimulation or even reduction. Both pH 2 and pH 8 extracts of the combined cultures of *A. glauca*$_{6776}$ and *P. parasitica* showed low concentration of UV-absorbing substances.

Thin layer chromatograms of *A. glauca* extracts exhibited two distinct kinds of bands: some blueish-white bands, white spots on the photographs, which are probably related to sterols or fatty acids and other UV-absorbing substances including the trisporoids, which appeared dark on the photographs (Figs. 4, 5) . This work is focusing on the latter.

Separation of the hitherto identified trisporoids by thin layer chromatography results in a pattern of distinct dark bands. In the present study, similar banding patterns were observed when culture extracts were subjected to the thin layer chromatography.

The combined culture extracts of *A. glauca* and *P. parasitica* showed some dark bands for trisporoids. The darkest bands were observed in the pH 2 and pH 8 combined culture extracts of *P. parasitica* (+) and *A. glauca*$_{101.48}$ (-) as well as in *P. parasitica* (-) and *A. glauca*$_{100.48}$ (+) at pH 2. The combined culture extract of the mated *A. glauca*$_{100.48}$ (+) and *A. glauca*$_{101.48}$ (-) also exhibited dark bands on the tlc plate (Fig. 4). The *A. glauca-P. parasitica* reaction is strongly mating type specific and *A. glauca* obviously interacts actively with the parasite. So in the interspecific complementary combinations, substance bands appeared that were not found in extracts of the single strains. No dark bands appeared in separations of the single and combined culture extracts of *A. glauca*$_{6776}$ (Fig. 5).

The homothallic species of *Absidia*: *A. orchidis*, *A. parricida* and *A. spinosa* exhibited the highest amount of UV-absorbing substances, including trisporoids, in pH 2 extracts at 285 nm. The substance concentrations were higher in the single culture extracts of *A. orchidis* and *A. parricida* than in *A. spinosa* in most cases. The interspecific interaction of *A. orchidis* and *P. parasitica* yielded similar amounts of trisporoids in most cases, whereas the interspecific combination of *A. parricida* and *P. parasitica* led to reduced production of trisporoids. The interspecific interaction of *A. spinosa* and *P. parasitica* (+) induced the production of trisporoids especially enriched at pH 8 (Table 6).

Both pH 2 and pH 8 single culture extracts of *A. spinosa* exhibited distinct three-component absorbance spectra for 4-dihydromethyl trisporate with maixima at 272, 282 nm and a shoulder at 294 nm (Fig. 36). Similar spectra with the same maxima were also detected in the pH 2 and pH 8 combined culture extracts of *A. spinosa* and *P. parasitica* (+) (Fig. 37). Spectra recorded for the single culture extracts of *A. orchidis* and *A. parricida* as well as in the interspecific combination with *P. parasitica* did not give evidence for the presence of such substances in considerable amounts.

The pH 2 single culture extracts of *A. orchidis* and *A. parricida* exhibited some relative dark bands possibly indicative to trisporoids. These bands showed the same migration distance as those in the pH 2 combined culture extracts of *P. parasitica* with either *A. orchidis* or *A. parricida.* These bands were also detected in the combined culture extracts of (+) and (-) *P. parasitica* at pH 2 (Fig. 6, 7). On the other hand, the culture extracts of *A. orchidis* and *A. parricida* also exhibited some of the white fluorescing spots on the tlc photographs probably related to sterols or fatty acids. In *A. spinosa* the presence of such substances was considerably reduced. *A. spinosa* probably produces only low concentrations of trisporoids as well as the dark bands on the tlc plate also appeared rather weak (Fig. 8).

Actinomucor elegans, *Blakeslea trispora* and *Chaetocladium brefeldii* are belonging to different families of Mucorales. The (+) culture of *B. trispora* produced a high amount of trisporic acid precursors. Compared with the absorbance values recorded for different members of Mucorales, *B. trispora* (+) is obviously the most active for the producion of trisporoids. Trisporic acid is normally synthesized cooperatively between the combined (+) and (-) cultures. Very high concentrations of trisporic acid compounds were detected in mated cultures of *B. trispora* in both pH 2 and pH 8 extracts (Table 7). The high levels of trisporoids in the culture extracts of *B. trispora* are also evident in the appearance of several dark bands on the analytical thin layer chromatogram (Fig. 10). So *B. trispora* is generally considered the most suitable organism for the production and identification as well as the characterization of trisporic acid substances.

The UV-absorbance spectra of the pH 8 culture extract of *B. trispora* (+) showed high absorbance in the trisporoid region with maxima at 235 and 284 nm (Fig. 40). A similar spectrum was also detected in the pH 2 extract with a single maximum at 283 nm (Fig. 40). On the other hand, the pH 2 mated culture extract of *B. trispora* exhibited a distinct absorbance spectrum with a 322 nm maximum (Fig. 41) typical for trisporic acid (Sutter 1970). A distinct precursor spectrum with maxima at 232 and 288 nm was also recorded for the pH 8 culture extract of mated *B. trispora* (Fig. 41).

Comparison of the tlc banding patterns obtained for the extracts of mated and unmated cultures of *B. trispora* as well as the combined cultures of *B. trispora* and *P. parasitica* showed variable levels of trisporoid biosynthesis. The darkest bands are obtained from the mated culture extracts of *B. trispora* at both pH 2 and pH 8 conditions (Fig. 10). Some dark bands are also appeared on the tlc plate for the pH 8 single culture extract of *B. trispora* (+). Only very weak bands were detected in the interspecific combinations of *B. trispora* and *P. parasitica* (Fig. 10).

Although the UV-absorbance values recorded for the single culture extracts of *Actinomucor elegans* and *Chaetocladium brefeldii* indicate a relatively low concentration of trisporoids, the pH 8 extract of *C. brefeldii* exhibited a single dark band on the tlc plate (Fig. 11). The interspecific combination of *C. brefeldii* and *P. parasitica* (+) highly stimulated the production of trisporoids and as a result very high concentration of trisporoids were found in the pH 2 extract with a maximum at 312 nm (Fig. 43). On the other hand, the pH 8 extract of the combined cultures of *C. brefeldii* and *P. parasitica* (+) exhibited the absorbance spectrum of 4-dihydromethyl trisporate with maxima at 272 nm, 282 nm and a shoulder at 293 nm as well as the pH 8 combined culture extract of *C. brefeldii* and *P. parasitica* (-) (Fig. 43).

Both pH 2 and pH 8 combined culture extracts of *P. parasitica* and *A. elegans* exhibited a distinct absorbance spectrum for 4-dihydromethyl trisporate with maxima at 272 nm, 282 nm and a shoulder at 293 nm (Fig. 39). Similar spectra were found in the single culture extracts of *A. elegans* (Fig. 38).

The pH 2 combined culture extract of *C. brefeldii* and *P. parasitica* (+) also showed a very distinct dark band on the tlc plate that was not found in the single strain extract. This distinct dark band has the same migration distance as one of the dark bands in the pH 8 mated culture extract of *B. trispora* (Fig. 11). No bands were observed in the separations of the single and combined culture extracts of *A. elegans* and *P. parasitica* (Fig. 9).

The data presented in tables (8, 9) show that the UV-absorbance values obtained from the spectrophotometric analysis of single culture extract of *L. macrospora* (Order: Kickxellales)

were higher than those measured for several Mucorales species including *C. elegans*, *G. persicaria*, *G. butleri* and *H. radiatus*.

The interspecific interaction of *L. macrospora* and *P. parasitica* induced the production of trisporoids enriched in the pH 2 extract. Generally, the amount of trisporoids produced in the pH 2 single culture extract of *L. macrospora* was higher than that obtained at pH 8 as well as in the interspecific combination of *L. macrospora* and *P. parasitica*.

Both pH 2 and pH 8 single culture extract of *L. macrospora* exerted high absorbance values and exhibited maxima at 278 and 271 nm in the pH 2 and pH 8 extracts, respectively (Fig. 50). The pH 2 combined culture extract of *L. macrospora* and *P. parasitica* exhibited a distinct trisporoid spectrum with a maximum at 310 nm (Fig. 51). The presence of such substances, in high concentration, in the single culture extract of *L. macrospora* as well as in the interspecific combinations with *P. parasitica* at pH 2 resulted in the separation of relatively dark bands on the tlc plate. These bands migrated the same distance as one of dark bands in the extract of mated cultures of *B. trispora* (Fig. 17).

Linderina macrospora was more active in the production of trisporoids than *Coemansia formosensis*. A high level of trisporoid-related absorbance could only be detected at 285 nm in *C. formosensis*. The secretion of such molecules in the culture medium of *C. formosensis* exhibited high absorbance in the specific region of trisporoids with a single maximum at 271 nm in the pH 8 extract (Fig. 44). The interspecific interaction of *C. formosensis* and *P. parasitica* reduced the production of trisporoids at 285 nm and increased it at 325 nm in most cases. The UV-absorbance spectra of the pH 8 combined culture extract of *C. formosensis* and *P. parasitica* (+) showed high absorbance in the specific region of trisporoids with a maximum at 272 nm (Fig. 44).

The tlc separation showed distinct dark bands in the pH 2 and pH 8 single culture extract of *C. formosensis* as well as in the combined culture extract of *C. formosensis* and *P. parasitica* (+) at pH 2 (Fig. 12).

Cunninghamella elegans and *Gilbertella persicaria* produced similar amounts of trisporoids. Generally, the amount of trisporoids measured at 285 nm was higher than that obtained at 325 nm for both pH 2 and pH 8 single culture extracts. The pH 8 single culture extract of *C. elegans* exhibited the distinct absorbance spectrum of 4-dihydromethyl trisporate with triple maxima at 272, 282 and 293 nm (Fig. 45). Such a spectrum was also recorded in the pH 8 combined culture extracts of *C. elegans* and *P. parasitica* (Fig. 46). The 4-dihydromethyl trisporate spectrum was observed in the pH 8 single culture extract of *G. persicaria* and also in the pH 8 combined culture extract of *G. persicaria* and *P. parasitica* (+) (Fig. 47).

The single culture extract of *C. elegans* as well as the pH 2 combined culture extract of *C. elegans* and *P. parasitica* (-) separated into some relatively dark bands on the tlc (Fig 13). In case of *G. persicaria*, dark bands were found in both pH 2 and pH 8 combined culture extracts of *G. persicaria* and *P. parasitica* (+) as well as in the combined culture extract of *G. persicaria* and *P. parasitica* (-) at pH 2 (Fig. 14).

Although the UV-absorbance values of the single culture extracts of *C. elegans* and *G. persicaria* are nearly similar, each organism behaved different in the interspecific combinations with *P. parasitica*. The interspecific interaction of *G. persicaria* and *P. parasitica* stimulated the production of trisporoids at both pH 2 and pH 8 conditions compared with the amount of trisporoids yielded in the single strain culture. In the interspecific interaction of *C. elegans* and *P. parasitica*, the high level of trisporoid stimulation was not observed and sometimes the production of trisporoids was depressed compared to the amount of trisporoids detected in the single culture extract of *C. elegans* (Table 8).

The UV-absorbance values of the single culture extracts of *G. butleri* and *H. radiatus* were higher at 285 nm than at 325 nm. The interspecific combinations of *G. butleri* and *P. parasitica* stimulated the production of trisporoids in most cases. Combination of *H. radiatus* and *P. parasitica* (+) enhanced the production of trisporoids found in the pH 2 extract. Enhancement was also found in the pH 8 combined culture extract of *H. radiatus* and *P. parasitica* (-) (Table 9).

The pH 8 single culture extract of *G. butleri* exhibited three-component spectra for 4-dihydromethyl trisporate with maxima at 272, 282 and 293 nm (Fig. 48). Such a spectrum was also observed in the pH 8 single culture extract of *H. radiatus* but it was not clear enough for peak detection (Fig. 49). In this respect, the pH 8 combined culture extracts of *G. butleri* and *P. parasitica* as well as the pH 8 combined culture extracts of *H. radiatus* and *P. parasitica* showed distinct absorbance spectra for 4-dihydromethyl trisporate with maxima at 272, 282 nm and a shoulder at 293 nm (Figs. 48, 49).

The single culture extracts of *G. butleri* and *H. radiatus* as well as the combinations extracts showed several weak dark bands for trisporoids on the tlc plate that might be related to the presence of several trisporoid substances in very low concentrations in the culture extracts of these fungi.

Six species of heterothallic *Mortierella* were analysed for their ability to produce trisporoids in the single cultures and in the combined cultures with *P. parasitica*. All *Mortierella* strains analysed have the ability to produce UV-absorbing substances at different levels (Tables 10-12). The UV-absorbance values of the pH 2 single culture extracts of *Mortierella* ranged from 4.3-15.5 and from 0.81-8.3 at 285 and 325 nm, respectively, whereas the absorbance of the pH 8 single culture extracts ranged from 3.3-11.9 and from 0.98-4.7 at 285 and 325 nm, respectively. These data indicate a higher concentration of UV-absorbing substances, including trisporoids, in the pH 2 extracts than at pH 8. The combined cultures of *Mortierella* and *P. parasitica* and the combined cultures of (+) and (-) strains of different *Mortierella* species showed UV-absorbance values for pH 2 extracts that ranged from 7.5-32.8 and from 0.98-13.9 at 285 and 325 nm, respectively, whereas in pH 8 extracts a range between 3.8-41.9 and from 0.06-22.4 at 285 and 325 nm, respectively was found. These ranges of absorbance values reflect the stimulation of the biosynthesis of UV-absorbing substances in different inter- and intraspecific combinations of *Mortierella* and *P. parasitica*.

Combination of cultures of *Mortierella* and *P. parasitica* exerted variable responses for the biosynthesis of UV-absorbing substances in different interspecific combinations. The combined cultures of *M. gamsii* and *P. parasitica* produced higher amounts of UV-absorbing

substances, including trisporoids, than that produced in the single culture extract. In the other *Mortierella* species, e.g *M. globulifera*, the absorbance values showed an irregular behaviour concerning the production of such substances in different interspecific combinations with *P. parasitica*. In the interspecific combinations of *M. parvispora* and *P. parasitica*, the biosynthesis of UV-absorbing substances was stimulated, but in other combinations the amount of such substances was reduced, e.g *M. humilis*, when compared to those obtained in *Mortierella* single culture extracts.

The majority of *Mortierella* single culture extracts did not exhibit distinct trisporoid absorbance spectra. The pH 2 single culture extracts of *M. humilis* (-) and *M. indohii* (+) showed a relatively high absorbance in the absorbance region of trisporoids with single maxima at 266 and 271 nm, respectively. The pH 8 single culture extract of *M. indohii* (-) had absorbance maximum at 269 nm.

The extracts of interspecific combinations of *Mortierella* and *P. parasitica* also did not exhibit distinct trisporoid absorbance spectra in most cases. The pH 8 combined culture extract of *M. minutissima* (+) and *P. parasitica* (-) exhibited a single absorbance maximum at 274 nm, whereas the pH 2 combined culture extracts of *M. minutissima* (-) with either *P. parasitica* (+) or *P. parasitica* (-) had a single absorbance maximum at 273 nm.

Only the pH 2 combined culture extract of (+) and (-) mating types of *M. minutissima* showed a relatively high absorbance in the specific region of trisporoids with a single maximum at 272 nm.

Comparison to the amount of UV-absorbing substances obtained in mated cultures of *B. trispora*, *Mortierella* generally produces much lower amounts of such substances than *B. trispora*. The amount of UV-absorbing substances produced in the mated cultures of different *Mortierella* species was less than 20 % of that produced by mated cultures of *B. trispora*. It is worth to mention, that the overall concentration of UV-absorbing substances is not directly corresponding to the concentration of trisporoids, especially in the *Mortierella* species that are also known to produce high amounts of sterols and fatty acids (e.g Weete and Gandhi 1999) with

similar absorbance characteristics. Ergosterol contents also was found to be increased in mated cultures and can be enhanced by addition of trisporic acid (Bu'Lock and Winstanley 1971; Gooday 1978).

The pH 8 combined culture extract of (+) and (-) mating types of *M. gamsii* and *M. globulifera* separated into relatively dark bands on the tlc plate (Figs. 18, 19). The pH 2 culture extract of *M. indohii* gave one dark band (Fig. 21).

Different *Mortierella* species exhibited different tlc banding patterns in the interspecific combinations with *P. parasitica*. In the pH 8 combined culture extract of *M. gamsii* (+) and *P. parasitica* (-) as well as the combined culture extract of *M. gamsii* (-) and *P. parasitica* (+) some dark bands possibly indicative to trisporoids appeared (Fig. 18). Both pH 2 and pH 8 combined culture extracts of *M. globulifera* (+) and *P. parasitica* (-) separated into dark bands (Fig. 19). The pH 8 combined culture extracts of *M. indohii* (-) and *P. parasitica* (+) gave relatively dark bands on the tlc plate as well as the pH 2 combined culture extract of *P. parasitica* (-) and *M. indohii* (+) (Fig. 21). The pH 8 extract of the combined cultures of *M. minutissima* (+) and *P. parasitica* (-) also showed a single dark band on the tlc plate (Fig. 22). No clear bands were observed for the combined culture extracts of *P. parasitica* with either *M. humilis* or *M. parvispora* (Figs. 20, 23).

The data presented in table (13) showed, that the amount of UV-absorbing substances produced in the single culture extract of *M. mucedo* (-) was higher than that produced in the (+) culture, whereas in *M. racemosus* the production of such substances was higher in the (+) single culture extract than in the (-) strain culture. The highest absorbance values were recorded in the pH 2 culture extract of *M. racemosus* (+).

The culture extracts of different single strains of *Mucor* showed different patterns of absorbance spectra in the absorbance region of trisporoids. Both pH 2 and pH 8 culture extracts of *M. mucedo* (-) exhibited distinct absorbance spectra for 4-dihydromethyl trisporate with maxima at 272, 282 nm and a shoulder at 293 nm (Fig. 52). The pH 2 and pH 8 culture extracts

of *M. racemosus* (+) also showed high absorbance in the specific region of trisporoids with single maxima at 276 and 274 nm for pH 2 and pH 8 extracts, respectively (Fig. 52).

The amount of UV-absorbing substances produced in mated cultures of *Mucor* was largely lower than that produced in mated cultures of *B. trispora* and contributed less than 10 % of the total amount produced in mated cultures of *B. trispora*.

In most cases, in the combined cultures of (+) and (-) mating types of *M. mucedo* and *M. racemosus* the production of trisporoids was not increased.

The single culture extracts of *M. racemosus* as well as the combined culture extracts of *M. racemosus* and *P. parasitica* showed very weak dark bands on the tlc plate that might be due to the presence of trisporoids in very low concentration in the culture extracts of these fungi (Fig. 25).

In the interspecific combinations of *M. mucedo* and *P. parasitica* the production of trisporoids was reduced, except in the pH 8 combined cultures of *P. parasitica* (+) and *M. mucedo* (-) which showed a higher absorbance value than the single culture extracts of *M. mucedo*. Combination of *M. racemosus* (+) and *P. parasitica* (-) also induced the production of trisporoids found in the pH 8 extract (Table 13).

The pH 2 combined culture extract of *M. mucedo* (+) and *P. parasitica* (+) showed absorbance maxima at 274 and 313 nm (Fig. 53). Both pH 2 and pH 8 combined culture extracts of *M. racemosus* (+) and *P. parasitica* (+) showed a high absorbance in the region of trisporoids with a single maximum at 280 nm (Fig. 55). A similar spectrum was recorded for the pH 8 combined culture extract of *M. racemosus* (+) and *P. parasitica* (-). The presence of 4-dihydromethyl trisporate in the combined culture extracts of *M. racemosus* and *P. parasitica* as well as in the combined cultures of (+) and (-) mating types of *M. racemosus* was observed but the spectra were not clear enough for peak resolution (Fig. 55).

The data obtained in table (14) reveal that the UV-absorbance values recorded for the single culture extracts of *M. africana* and *P. blakesleeanus* showed different concentrations of UV-absorbing substances including trisporoids. *P. blakesleeanus* was more active in the production of such substances than several members of Mucorales including the already mentioned *M. africana*. Generally, the amount of trisporoids measured at 285 nm was higher than that recorded at 325 nm. On the other hand, the pH 2 single culture extract of *M. africana* contained a higher amount of trisporoids than the pH 8 extract.

The pH 8 single culture extracts of (+) and (-) strains of *P. blakesleeanus* showed distinct 4-dihydromethyl trisporate spectra with maxima at 272 and 282 nm and a shoulder at 293 nm (Fig. 56). The pH 2 single culture extract of (+) and (-) *P. blakesleeanus* also exhibited high absorbance in the absorbance region of trisporoids with a single maximum at 271 nm (Fig. 56). On the other hand, the spectra of 4-dihydromethyl trisporate was observed in the pH 8 single culture extract of *M. africana* as well as in the pH 8 combined culture extract of *M. africana* and *P. parasitica* (+) but the spectra were not clear enough for peak detection.

Combination of (+) and (-) cultures of *P. blakesleeanus* did not improve the production of trisporoids. The amount of UV-absorbing substances produced in mated cultures of *P. blakesleeanus* was lower than that produced in mated cultures of *B. trispora* and contributed less than 25 % of the overall amount produced in mated cultures of *B. trispora.* Corresponding to the amount of UV-absorbing substances produced in mated cultures of *Mucor*, *P. blakesleeanus* produced two to three times as much than that produced in *Mucor*.

The interspecific combinations of *P. blakesleeanus* and *P. parasitica* did not increase the production of trisporoids in most combinations compared to the amount of trisporoids produced in single culture extracts of *P. blakesleeanus*. Trisporoid production in the combined culture extracts of *M. africana* and *P. parasitica* was decreased compared to that obtained in the single culture extract of *M. africana* except in pH 8 extract of *P. parasitica* (+) and *M. africana* where an increase was detected (Table 14).

Several extracts of the combined cultures of *P. blakesleeanus* and *P. parasitica* exhibited distinct absorbance spectra for 4-dihydromethyl trisporate with maxima at 272 and 282 nm and a shoulder at 293 nm (Fig. 57). Another trisporoid spectrum with a single maximum at 272 nm was also observed in the pH 2 combined culture extract of *P. parasitica* (+) and *P. blakesleeanus* (+) (Fig. 57).

The darkest bands were observed on the tlc plate for the pH 8 single culture extracts of (+) and (-) *P. blakesleeanus*. A similar band with the same migration distance was also detected in the pH 8 combined culture extract of *P. blakesleeanus* (+) and *P. parasitica* (-) as well as in the pH 8 combined culture extract of (+) and (-) strains of *P. blakesleeanus* (Fig. 57). Some weak bands were also observed in the pH 2 single culture extracts of (+) and (-) strains of *P. blakesleeanus* as well as in the combined cultures of (+) and (-) mating types of *P. blakesleeanus* at pH 2 (Fig. 27). Only a few weak bands were appeared on the tlc plate of *M. africana*, possibly due to the presence of trisporoid substances only in very low concentration in the extracts (Fig. 26).

The data obtained in table (15) show the UV-absorbance values measured for the culture extracts of three homothallic species: *Pilaira anomala*, *Syncephalastrum racemosum* and *Syzigites megalocarpus*. The UV-absorbance values measured at 325 nm for the single culture extracts of these fungi are nearly similar. At 285 nm, the UV-absorbance values recorded were relatively higher in the single culture extract of *S. megalocarpus* than in the single culture extracts of *P. anomala* and *S. racemosum*.

The UV-absorbance spectra obtained for the single culture extracts of these fungi indicate different levels of trisporoid production. The most distinct spectrum for 4-dihydromethyl trisporate, with maxima at 272, 281 nm and a shoulder at 293 nm, was detected in the pH 8 single culture extract of *S. megalocarpus* (Fig. 60). 4-dihydromethyl trisporate was also found in the pH 8 single culture extract of *S. racemosum* and pH 2 culture extract of *S. megalocarpus* (Fig. 60) but the spectra were not clear enough to allow peak differentiation.

The pH 2 single culture extract of *S. racemosum* exhibited dark bands on the tlc that were not found in separations of *P. anomala* and *S. megalocarpus* single culture extracts.

In combined cultures of *P. anomala* and *P. parasitica* the biosynthesis of trisporoids was stimulated and as a result, high amounts of trisporoid substances were detected in both pH 2 and pH 8 extracts. In this respect, for the interspecific combinations of *P. parasitica* with either *S. racemosum* or *S. megalocarpus* lower amounts for the production of trisporoids were calculated in most cases compared to those obtained in the single culture extracts of these fungi (Table 15).

Typical absorbance spectrum for 4-dihydromethyl trisporate with triple maxima at 272, 282 and 293 nm was detected in the pH 8 combined culture extract of *P. anomala* and *P. parasitica* (+) as well as in the pH 8 combined culture extract of *P. anamala* and *P. parasitica* (-) (Fig. 59). Another trisporoid spectrum with maxima at 271, 283, 296 and 308 nm was recorded in the pH 2 combined culture extract of *P. anomala* and *P. parasitica* (+) (Fig. 59). The pH 2 combined culture extract of *P. anomala* and *P. parasitica* (-) exhibited distinct trisporoid spectrum with maxima at 283 and 313 nm (Fig. 59). The pH 2 combined culture extract of *S. racemosum* and *P. parasitica* (+) as well as the pH 2 combined culture extract of *S. racemosum* and *P. parasitica* (-) exhibited high absorbance in the trisporoid region with single maxima at 273 and 270 nm, respectively. The 4-dihydromethyl trisporate spectrum was also observed in the pH 2 and pH 8 combined culture extracts of *S. megalocarpus* and *P. parasitica* (+) (Fig. 61) as well as in the single culture extracts of *S. megalocarpus* (Fig. 60). The 4-dihydromethyl trisporate spectra of the pH 8 extracts exhibited maxima at 272, 281 nm and a shoulder at 293 nm (Figs. 60, 61) but the spectra in the pH 2 extracts were not clear enough for peak identification (Figs. 60, 61) .

The tlc pattern for the pH 2 combined culture extract of *P. anomala* and *P. parasitica* (+) and the combined culture extract of *P. anomala* and *P. parasitica* (-) showed distinct dark bands. These bands were not found in the single culture extracts of *P. anomala* (Fig. 28). The tlc chromatograms of the pH 2 combined culture extract of *S. racemosum* and *P. parasitica* (+) showed the same dark bands on the tlc plates as the pH 2 combined culture extract of *S.*

racemosum and *P. parasitica* (-). These dark bands are similar to those obtained in the single culture extract of *S. racemosum* at pH 2 and in the pH 2 combined culture extract of (+) and (-) strains of *P. parasitica* (Fig. 29). The tlc plates of *P. anomala*, *S. megalocarpus* and *S. racemosum* showed several weak bands for different culture extracts, possibly due to the presence of trisporoid compounds in very low concentrations in the culture extracts of these fungi.

The data presented in table (16) show the UV-absorbance values recorded for the culture extracts of two homothallic mucoraceous fungi, namely *Thamnostylum piriforme* and *Zygorhynchus moelleri* and the heterothallic *Thamnidium elegans*.

The UV-absorbance values measured at 285 and 325 nm for the single culture extracts of these strains showed variable levels of trisporoid production. Generally, the amount of trisporoids measured at 285 nm was higher than that determined at 325 nm in most cases. The amount of trisporoids produced in the single culture extracts of (+) and (-) strains of *T. elegans* at 285 nm was higher than that obtained in the single culture extracts of several other members of mucoralean fungi including *T. piriforme* and *Z. moelleri*. The highest amount of trisporoids was recorded in the pH 2 single culture extracts of (+) and (-) strains of *T. elegans* at 285 nm as well as in the combined culture extract of (+) and (-) mating types of *T. elegans*.

The UV-absorbance spectra of the single culture extracts of these fungi showed different levels of trisporoid production. The most distinct trisporoid spectra with a single maximum at 285 nm was recorded in the pH 2 single culture extracts of (+) and (-) strains of *T. elegans* (Fig. 62). The presence of 4-dihydromethyl trisporate in the pH 8 culture extract of *T. elegans* (+) was also observed (Fig. 62).

The single culture extracts of *Z. moelleri* showed relatively high absorbance in the specific region of trisporoids with a single maximum at 273 nm, whereas the single culture extracts of *T. piriforme* were less active in this respect.

The tlc's of single culture extracts of *T. elegans*, *T. piriforme* and *Z. moelleri* showed several weak bands possibly indicative to trisporoids (Figs. 31-33).

The interspecific combinations of *T. elegans* and *P. parasitica* did not induce the production of trisporoids and sometimes reduced the production of UV-absorbing substances when compared to those produced in single culture extracts of *T. elegans*. On the other hand, the trisporoid production in the combined culture extracts of (+) and (-) mating types of *T. elegans* was higher than that produced in the single culture of *T. elegans* at 285 nm (Table 16).

Combination of cultures of *T. piriforme* and *P. parasitica* induced the production of trisporoid in most cases compared to the amount of trisporoids produced by the single *T. piriforme*. The induction effect was observed more often in pH 8 than in pH 2 extracts. The combination of cultures of *T. piriforme* and *P. parasitica* (-) induced higher amount of trisporoids than the combined cultures of *T. piriforme* and *P. parasitica* (+). There is no great difference in the production of trisporoids in the combined culture extracts of *Z. moelleri* and *P. parasitica* compared to the amount of trisporoids produced in the single culture extract of *Z. moelleri* (Table 16).

The pH 2 and pH 8 combined culture extracts of *P. parasitica* (+) with either (+) or (-) strains of *T. elegans* exhibited distinct absorbance spectra for 4-dihydromethyl trisporate with maxima at 272, 282 nm and a shoulder at 293 nm (Fig. 63). In this respect, the pH 2 combined culture extract of *T. elegans* (+) and *P. parasitica* (-) as well as the pH 2 combined culture extract of *T. elegans* (-) and *P. parasitica* (-) exhibited high absorbance in the specific region of trisporoids with a single maximum at 282 and 271 nm, respectively (Fig. 64). The pH 2 combined culture extract of (+) and (-) mating types of *T. elegans* exhibited a high absorbance in the absorbance region of trisporoids with a single maximum at 288 nm (Fig. 64).

The UV-absorbance spectra of the combined culture extracts of *T. piriforme* and *P. parasitica* as well as the combined cultures of *Z. moelleri* and *P. parasitica* showed only low concentrations of trisporoids.

The tlc's of combined culture extracts of *P. parasitica* and *T. elegans* as well as the combined culture extracts of *P. parasitica* with either *T. piriforme* or *Z. moelleri* showed only weak trisporoid bands (Figs. 31-33).

The majority of zygomycetes extracts exhibited several weak dark bands, possibly indicative to trisporoids, on the tlc plates. This phenomenon reflects the presence of minor trisporoid substances in very low concentrations in these extracts.

3.2. Analysis of thin layer chromatography fractions

To have more evidence to the identification of the tlc trisporoid bands, several of dark bands separated from different zygomycetes culture extracts were individually removed from the tlc plates and eluted into ethanol. The spectrophotometric analysis of these single bands resulted in typical absorbance spectra for trisporoid compounds.

The analysis of a tlc band recovered from both pH 2 and pH 8 single culture extracts of *P. parasitica* (-) had typical absorbance spectra for trisporin or trisporol with a single maximum at 301 and 302 nm for pH 2 and pH 8 extracts, respectively (Fig. 65). A single band recovered from both pH 2 and pH 8 combined culture extracts of (+) and (-) *P. parasitica* showed the 4-dihydromethyl trisporate spectrum with triple maxima at 272, 282 and 294 nm (Fig. 65).

The tlc band of the pH 2 combined culture extract of *C. brefeldii* and *P. parasitica* (+) also exhibited an absorbance spectrum similar to those of trisporin or trisporol with maxima at 240 and 306 nm (Fig. 66).

The tlc band obtained from the pH 2 single culture extract of *C. formosensis* had a single maximum at 269 nm (Fig. 67). The analysis of another two single bands from pH 8 extract, migrating fast and slow on the silica gel, exhibited maxima at 275 and 271 nm, respectively (Fig. 67).

The tlc band of the pH 2 single culture extract of *L. macrospora* exhibited a high absorbance in the absorbance region of trisporoids with maxima at 238 and 274 nm (Fig. 68). The analysis of the corresponding band in the pH 2 combined culture extract of *L. macrospora* and *P. parasitica* (+) resulted in a spectrum showing maxima at 283 and 294 nm, whereas the band from the pH 2 combined culture extract of *L. macrospora* and *P. parasitica* (-) had a single maximum at 283 nm (Fig. 68).

The analysis of a tlc band obtained in the pH 8 single culture extract of (+) and (-) *P. blakesleeanus* showed the typical absorbance spectrum of 4-dihydromethyl trisporate with triple maxima at 272, 282 and 294 nm (Fig. 70). The spectrum of another single band migrating more

slowly on the silica gel also indicative to trisporoids and exhibiting a single maximum at 283 nm (Fig. 70). The analysis of a band obtained in the pH 8 combined culture extract of *P. blakesleeanus* (+) and *P. parasitica* (-) as well as one from the pH 8 combined cultures of (+) and (-) *P. blakesleeanus* showed typical absorbance spectra for 4-dihydromethyl trisporate with triple maxima at 272, 282 and 294 nm (Fig. 71). The analysis of a single band migrating slower on the silica gel for the same combinations also exhibited high absorbance in the specific region of trisporoids with a single maximum at 283 nm (Fig. 71). The spectra recorded for single bands in the interspecific combination of *P. blakesleeanus* (+) and *P. parasitica* (-) as well as from the combined cultures of (+) and (-) mating types of *P. blakesleeanus* were identical with the corresponding bands obtained from the pH 8 single culture extracts of (+) and (-) strains of *P. blakesleeanus* (Figs. 70, 71).

The analysis of a tlc band obtained from the pH 2 combined culture extract of *P. anomala* and *P. parasitica* (+) as well as one from the pH 2 combined cultures of *P. anomala* and *P. parasitica* (-) resulted in typical trisporin / trisporol absorbance spectra with a single maximum at 303 nm (Fig. 69).

3.3. Biological activity of trisporoids

Among several culture extracts that were analysed and bioassayed for the induction of zygophores in both (+) and (-) strains of *M. mucedo*, *B. trispora* culture extracts were the most active for the induction of zygophores in both (+) and (-) strains of *M. mucedo*. The single culture extracts of *B. trispora* stimulate zygophore production in both (+) and (-) strains of *M. mucedo* (Table 18)

Data obtained from the bioassay of *B. trispora* culture extracts showed that the (-) strain of *M. mucedo* is more sensitive towards stimulation than the (+) strain, so the bioassay generally showed more zygophores in the (-) strain of *M. mucedo* than in the (+) strain. Single culture extracts of *B. trispora* (+) induced more zygophores in both (+) and (-) strains of *M. mucedo* than in the (-) strain extracts. Both pH 2 and pH 8 extracts obtained from the combined cultures of (+) and (-) mating types of *B. trispora* induced the highest numbers of zygophores in both (+) and (-) strains of *M. mucedo* (Table 18).

B. trispora and *P. parasitica* combination extracts showed different levels of zygophore induction in (+) and (-) strains of *M. mucedo*. Both pH 2 and pH 8 combined culture extracts of *B. trispora* (-) and *P. parasitica* (+) exerted the highest level of zygophore induction in both (+) and (-) strains of *M. mucedo*. The combined cultures of *B. trispora* (+) and *P. parasitica* (+) induced average numbers of 53.5 and 39.25 zygophores in *M. mucedo* (-) at pH 2 and pH 8 extracts, respectively, while the combined cultures of *B. trispora* (+) and *P. parasitica* (-) induced 61.5 and 93.5 zygophores in *M. mucedo* (-) at pH 2 and pH 8 extracts, respectively. The combined culture extracts of *B. trispora* (-) and *P. parasitica* (-) showed very low activity for zygophore induction in *M. mucedo* (-) and no response at all in the (+) type (Table 18).

Single culture extracts of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ induced *M. mucedo* (-) to produce zygophores, but no zygophores were observed in *M. mucedo* (+). The single culture extracts of *A. glauca*$_{100.48}$ induced *M. mucedo* (-) to produce average numbers of 61.5 and 35.75 zygophores for pH 2 and pH 8 extracts, respectively, whereas averages of 54.25 and 27.5 zygophores were produced for pH 2 and pH 8 single culture extracts of *A. glauca*$_{101.48}$. Single culture extracts of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ induced more zygophores in *M. mucedo* (-

) than the single culture extracts of *A. glauca*$_{6776a}$ and *A. glauca*$_{6776b}$. The combined culture extracts of (+) and (-) mating types of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ stimulate zygophore production in *M. mucedo* (-) and the degree of induction was higher in the pH 2 extract (116 zygophores) than at pH 8 (54.75). In this respect, also the combined culture extracts of (+) and (-) *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ were more active in inducing zygophores in *M. mucedo* (-) than the extracts obtained from the combined cultures of (+) and (-) mating types of *A. glauca*$_{6776}$ at both pH 2 and pH 8 (Table 17).

The combined culture extracts of *A. glauca*$_{100.48}$ (+) and *P. parasitica* (-) induced the highest numbers of zygophores (124.5 and 112 at pH 2 and pH 8 extracts, respectively) in *M. mucedo* (-) without any response in *M. mucedo* (+). Averages of 92.5 and 68.25 zygophores were also obtained in *M. mucedo* (-) for the combined cultures of *P. parasitica* (+) and *A. glauca*$_{101.48}$ (-) at pH 2 and pH 8 extracts, respectively, while the combined cultures of *P. parasitica* (+) and *A. glauca*$_{100.48}$ (+) induced 89.5 and 55.75 zygophores in *M. mucedo* (-) at pH 2 and pH 8 extracts, respectively. The combined culture extracts of *P. parasitica* (-) and *A. glauca*$_{101.48}$ (-) showed very low activity concerning zygophore induction in *M. mucedo* (-) and gave no response at all in the (+) culture. The combined culture extracts of *A. glauca*$_{6776}$ and *P. parasitica* also showed very low activity for zygophore induction in *M. mucedo* (-) without any positive response in *M. mucedo* (+) (Table 17). On the other hand, both single and combined culture extracts of the remaining strains (37 strains) of zygomycetes gave negative responses for the induction of zygophores in both (+) and (-) strains of *M. mucedo*.

3.4. Extent of parasitic interactions of *Parasitella parasitica* with different hosts

Forty three homothallic and heterothallic strains of zygomycetes were grown in combination with (+) and (-) strains each of *P. parasitica* on supplemented minimal agar medium in Petri dishes. Typical infection structures could be detected between the aerial mycelia of several hosts and the parasite after 4-6 days of inoculation (Table 19).

Forteen strains showed strong parasitic interaction with the formation of several sikyospores with *P. parasitica* (+), whereas eleven strains showed equally strong interactions with *P. parasitica* (-). Little interaction was observed in four members of zygomycetes with *P. parasitica* (+) while five strains also exhibited such reaction with *P. parasitica* (-). The infrequent interactions of these strains occured with or without sikyospore formation. Twenty five strains of zygomycetes showed no reaction in crosses with *P. parasitica* (+), while twenty seven strains exhibited no reaction with *P. parasitica* (-).

The heterothallic species *A. glauca* showed a strongly mating type specific reaction. Strong parasitic interaction with the formation of several sikyospores occurred with the complementary mating types of *P. parasitica* (+) (Figs. 72, 75) and no reaction was observed within combinations of identical mating types. Generally, the strength of parasitic interactions of *P. parasitica* was highly different between the two mating types. The (+) strain of *P. parasitica* showed strong parasitic interaction with the formation of several sikyospores with the complementary mating types of *A. glauca* while the *P. parasitica* (-) showed little interaction with the complementary mating types of *A. glauca* (Table 19).

Among the homothallic strains analysed, *L. macrospora* behaved differently with the (+) and (-) strains of *P. parasitica*. *L. macrospora* gave strong parasitic interaction with the formation of several sikyospores with *P. parasitica* (-) (Figs. 85, 86), but little interaction without any sikyospores was observed with *P. parasitica* (+). The reverse occurred in case of the homothallic *C. brefeldii*. *C. brefeldii* exhibited strong parasitic interaction with the formation of several sikyospores with *P. parasitica* (+) (Fig. 81), whereas the cultivation with *P. parasitica* (-) showed little interaction without any sikyospore formation.

Both (+) and (-) strains of *P. parasitica* showed strong interaction with complete parasitic development and a high number of sikyospores formed with several hosts, belonging to both homothallic and heterothallic species, including *A. parricida* (Figs. 76, 77), *B. trispora* (+), *B. trispora* (-) (Figs. 79, 80), *G. persicaria* (Figs. 82-84), *M. mucedo* (-), *M. racemosus* (+) (Figs. 90, 91), *M. racemosus* (-) (Figs. 92-95), *M. africana* (Figs. 96-99), *T. elegans* (+) (Figs. 100, 101) and *Z. moelleri* (Fig. 104). *T. elegans* (-), on the other hand showed strong parasitic interaction with *P. parasitica* (+) (Fig. 101) and little interaction with *P. parasitica* (-) (Fig. 103), but these interactions nevertheless led to sikyospore formation. All *Mortierella* species did not undergo any reactions with both (+) and (-) strains of *P. parasitica* (Figs. 107, 108). The same was true for several members of homothallic and heterothallic zygomycetes, e.g *A. orchidis* (Fig. 105), *A. spinosa* (Fig. 106), *C. formosensis*, *C. elegans*, *H. radiatus*, *P. blakesleeanus*, *P. anomala* (Fig. 109), *S. racemosum*, *S. megalocarpus* and *T. piriforme*.

Correlating the measurements of trisporoids in the culture extracts of different zygomycetes in relation to the strength of their parasitic interactions indicates, that most of those zygomycetes showing strong parasitic interaction are also producing considerable amounts of trisporoids. In the interspecific interactions of *P. parasitica* and *A. glauca*, the production of trisporoid compounds is sometimes stimulated. This stimulatory effect was observed in all combinations of *A. glauca*$_{100.48}$ or *A. glauca*$_{101.48}$ with *P. parasitica* in the pH 2 extract. Although the homothallic *A. parricida* exhibited equally strong parasitic interactions with (+) and (-) strains of *P. parasitica*, the production of trisporoids was reduced in the interspecific combinations with *P. parasitica* when compared to the amount of trisporoids produced in the single strain. *G. persicaria*, which exhibited strong interaction with (+) and (-) *P. parasitica*, produced higher amounts of trisporoids in the interspecific combinations with *P. parasitica* than in the single culture. The induction of trisporoid production in the host-parasite system was not found in case of *B. trispora*. *B. trispora* produced high amounts of trisporoids in the mated cultures but not in the interspecific combinations with *P. parasitica*. The (+) culture of *B. trispora* also contained a high amount of trisporic acid precursors. *B. trispora* showed strong parasitic interaction with (+) and (-) strains of *P. parasitica*. The homothallic *C. brefeldii*, which exhibited strong parasitic interaction with *P. parasitica* (+), produced a high amount of trisporoids in the interspecific combination of *C. brefeldii* and *P. parasitica* (+) at pH 2. *L.*

macrospora, which exhibited strong parasitic interaction with *P. parasitica* (-), also produced a high amount of trisporoids in the interspecific combinations with *P. parasitica* at pH 2. In *M. racemosus* which exhibited strong parasitic interaction with (+) and (-) *P. parasitica*, a considerable amount of trisporoids was formed in both single strains and in combined cultures with *P. parasitica*. The concentration of trisporoids in the interspecific combinations of *M. racemosus* and *P. parasitica* generally varied between different combinations. *M. africana* and *T. elegans* did not show an increase in the production of trisporoids in their interspecific combinations with *P. parasitica* in most cases. On the other hand, the single culture of *T. elegans* produced a considerable amount of trisporic acid precursors. *Z. moelleri*, which showed strong parasitic interaction with *P. parasitica*, exhibited a slight increase in the production of trisporoids in the interspecific combinations with *P. parasitica* in some cases in pH 8 extracts compared to those produced in the single culture of *Z. moelleri*.

Zygospore formation was observed in combined cultures of (+) and (-) strains of several heterothallic species. Many species of zygomycetes were able to produce zygospores on the solidified supplemented minimal medium. These species belong to many genera including *Absidia*, *Blakeslea*, *Mortierella*, *Mucor*, *Parasitella* and *Thamnidium*. The (+) and (-) mating types of *P. blakesleeanus* showed incomplete sexual reaction with the formation of conjugated progametangia only. Three species of *Mortierella* including M. *humilis*, *M. indohii* (Fig. 112) and *M. parvispora* were able to produce zygospores, whereas the other three species of *Mortierella*: *M. gamsii*, *M. globulifera* and *M. minutissima* did not form zygospores under the conditions employed in this work (Table 20). *P. parasitica* was sexually active. Sexual interaction and zygospore formation between (+) and (-) strains of this fungus was detected on the supplemented minimal medium (Figs. 113, 114). The ability of some homothallic species of zygomycetes to produce zygospores under these conditions was also observed, especially in the homothallic *A. parricida* (Fig. 111) and *Z. moelleri*.

4. DISCUSSION

All zygomycetes fungi were growed on the supplemented minimal medium containing maltose as a carbon source, because under these conditions the highest rates of parasitic infection are obtained (Burmester and Wöstemeyer 1994). Glucose tends to suppress the parasitic growth. Trisporoids were extracted from the culture filtrates of both single strains and combination cultures with *P. parasitica* in two steps, yielding an acid and an alkaline to neutral fractions.

Absidia glauca single culture extracts exhibited different levels of UV-absorbing substances biosynthesis, including trisporoids. The highest amount of these substances were recorded in *A. glauca*$_{6776b}$ (-) extracts. The interspecific combination of *A. glauca*$_{100.48}$ or *A. glauca*$_{101.48}$ with *P. parasitica* stimulated the production of UV-absorbing substances, including trisporoids, at pH 2. Similar reaction was also detected in the combined cultures of (+) and (-) mating types of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ respectively. *A. glauca-P. parasitica* reaction is strongly mating type dependent, so in the interspecific complementary combinations of *A. glauca*$_{100.48}$ or *A. glauca*$_{101.48}$ with *P. parasitica* substance bands appeared on the thin layer chromatography plate that were not found in the single culture extracts of these fungi. The mated culture extracts of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ also exhibited dark bands on the thin layer chromatography. Recently, Wöstemeyer *et al.* (1995, 1997 a, b) extensively studied the host-parasite system *A. glauca-P. parasitica*. Among the most important observations, that the trisporic acid seems to be involved in mediating the recognition between *P. parasitica* and *A. glauca*. They also reported that there are analogies between the sexual and parasitic interactions of *A. glauca* and *P. parasitica*. The cocultures of *P. parasitica* mating types produced only trace amount of trisporic acid, whereas the complementary combinations of *P. parasitica* and *A. glauca* increased the production of trisporic acid considerably. To prove the role of trisporic acid signalling system in both sexuality and parasitism, one of the key enzymes in the biosynthesis pathway of trisporic acid, 4-dihydromethyl trisporate dehydrogenase, has been isolated and biochemically characterized and its gene has been identified and cloned in *Mucor mucedo* (Czempinski *et al.* 1996).

The homothallic species of *Absidia*: *A. orchidis*, *A. parricida* and *A. spinosa* produced the highest amounts of UV-absorbing substances, including trisporoids, in the pH 2 extract and

measured only at 285 nm. The amount of these substances were higher in the single culture extracts of *A. orchidis* and *A. parricida* than in *A. spinosa* in most cases. Not all the concentration of UV-absorbing substances related to the trisporoids, because some white spots probably related to sterol or fatty acids, were also formed on the thin layer chromatography of different *Absidia* extracts as well as in different *Mortierella* species extracts. Such substances were reduced in *A. spinosa* extracts. On the other hand, the pH 2 and pH 8 single culture extracts of *A. spinosa* exhibited distinct absorbance spectra for 4-dihydromethyl trisporate with maxima at 272, 282 nm and a shoulder at 294 nm as well as in the combined culture extracts of *A. spinosa* and *P. parasitica* (+). Such spectra not found in case of *A. orchidis* and *A. parricida.* The three-components spectrum of 4-dihydromethyl trisporate was common among several culture extracts analysed. The 4-dihydromethyl trisporate spectrum was detected in the single culture extracts of several members of homothallic and heterothallic mucoralean fungi including *Chaetocladium brefeldii* (Chaetocladiaceae), *Cunninghamella elegans* (Cunninghamellaceae), *Gilbertella persicaria* (Gilbertellaceae), *Gongronella butleri* (Mucoraceae), *Halteromyces radiatus* (Mucoraceae), *Mucor mucedo* and *M. racemosus* (Mucoraceae), *Phycomyces blakesleeanus* (Phycomycetaceae), *Syzigites megalocarpus* (Mucoraceae) and *Thamnidium elegans* (Thamnidiaceae) as well as in the combined culture extracts of these fungi and *P. parasitica*. On the other hand, the analysis of tlc single bands recovered from the combined culture extracts of (+) and (-) mating types of *P. parasitica* exhibited the spectrum of 4-dihydromethyl trisporate with triple maxima at 272, 282 and 294 nm, whereas the tlc bands isolated from the single culture extracts of *P. parasitica* (-) exhibited the absorbance spectrum of trisporin / trisporol compounds with a single maxima at 301 and 302 nm for pH 2 and pH 8 extracts, respectively. Sutter and Whitaker (1981 b) reported that 4-dihydromethyl trisporate in the (+) culture extract of *Blakeslea trispora* migrated as a single spot on the thin layer chromatography and exhibited UV-absorbance maxima at 276, 283 and 296 nm. The (-) culture extracts of *M. mucedo* and *B. trispora* on the other hand contained two biologically active fractions, trisporin B and C (Nieuwenhuis and van den Ende 1975; Sutter 1987). These substances exhibited UV-absorbance maxima at 231 and 300 nm in the culture extract of *B. trispora* (Sutter and Zawodny 1984).

It is evident that, *Blakeslea trispora* is the most active trisporoid producer among several heterothallic species within the Mucorales. Very high amount of trisporic acid precursors was detected in *B. trispora* (+) at pH 8 extract. Trisporic acid is normally synthesized cooperatively between (+) and (-) mating types. The mated cultures of *B. trispora* produced very high amount of trisporic acid compounds at both pH 2 and pH 8 extracts. The pH 2 extract exhibited the absorbance spectrum of trisporic acid with a maximum at 322 nm while the pH 8 extract had a distinct absorbance spectrum for trisporic acid precursors with maxima at 232 and 288 nm. Trisporic acid B and C are the major trisporoid compounds in mated cultures of *B. trispora* and enriched in the acid fraction (van den Ende 1968; Sutter 1970; Sutter *et al.* 1973). The neutral fraction of mated cultures of *B. trispora* exhibited a UV-absorbance maximum at 285 nm (Sutter 1970).

On the other hand, the presence of trisporoids in very high concentrations in mated culture extracts of *B. trispora* as well as in the single culture extract of *B. trispora* (+) separated into several dark bands on the thin layer chromatography. Sutter *et al.* (1973) reported that trisporic acids B and C were resolved by silica gel thin layer chromatography with similar Rf values. These compounds also exhibited identical UV-absorbance spectra and the same biological activity in the *Mucor mucedo*. A partially purified extract isolated from the (+) culture of *B. trispora* was resolved into 7 components by Sephadex LH-20 chromatography (Sutter and Whitaker 1981 b).

The high concentration of trisporoids was also detected in the pH 2 combined cultures of *Chaetocladium brefeldii* and *Parasitella parasitica* (+), whereas the single culture extract of *C. brefeldii* and *P. parasitica* showed lower concentrations for these substances. The production of trisporic acid in the pH 2 combined cultures of *C. brefeldii* and *P. parasitica* (+) could be detected but the presence of other compounds in the culture extract led to shift the maximum somewhat to lower wavelength. The analysis of a distinct single band obtained on the thin layer chromatography for the same combination exhibited an absorbance spectrum similar to those for trisporin / trisporol compounds with maxima at 240 and 306 nm. Trisporin and trisporol compounds in the (-) culture extract of *B. trispora* exhibited a maximum absorbance at 231 and 300 nm (Sutter and Zawodny 1984).

Coemansia formosensis and *Linderina macrospora* (order: Kickxellales) were more active for the production of trisporoids than several members of Mucorales and Mortierellales. The single culture extract of *C. formosensis* produced high amount of trisporoids measured only at 285 nm. Both pH 2 and pH 8 extracts of *C. formosensis* showed distinct dark bands on the thin layer chromatography as well as in the pH 2 combined culture extract of *C. formosensis* and *P. parasitica* (+). *L. macrospora* was more active for the production of trisporoids than *C. formosensis*. Very high concentration of these substances was detected in the pH 2 single culture extract of *L. macrospora* as well as in the interspecific combinations of *L. macrospora* and *P. parasitica*. The high concentration of trisporoids in the pH 2 single culture extract of *L. macrospora* exhibited dark bands on the thin layer chromatography and an absorbance maximum at 278 nm. In the absorbance spectra of the pH 2 combined culture extracts of *L. macrospora* and *P. parasitica*, the formation of trisporic acid could be detected but due to the presence of other compounds in the culture extracts the absorbance maximum was somewhat shifted towards lower wavelength. On the other hand, the analysis of single bands isolated from the pH 2 combined culture extracts of *L. macrospora* and *P. parasitica* exhibited trisporic acid similar spectra, while the analysis of single bands isolated from pH 2 and pH 8 single culture extracts of *C. formosensis* had spectra similar to those for trisporic acid precursors.

Six species of *Mortierella* were also analysed for the production of trisporoids. Generally, the production of UV-absorbing substances was varied between different species of *Mortierella*. It is worth to mention that, not the overall concentration of UV-absorbing substances related to trisporoids because some white fluorescing spots, probably related to sterols or fatty acids, are formed on the thin layer chromatography for different *Mortierella* species extracts. *Mortierella* species are also known to produce high amounts of sterols and fatty acids (e.g Weete and Gandhi 1999) with the same absorbance characteristics. *Mortierella* species also produced low amounts of trisporoids as in members of Mucorales, including *C. brefeldii*, *M. africana*, *P. anomala* and *Z. moelleri*, so in the UV-absorbance spectra of several *Mortierella* extracts the indication for the presence of trisporoids in high concentration not found. Most of these single culture extracts did not exhibit distinct trisporoid absorbance spectra as well as in several combination extracts with *P. parasitica*. The most distinct absorbance spectra for trisporoids were detected in the pH 2 single culture extracts of *M.*

humilis (-) and *M. indohii* (+) with maxima at 266 and 271 nm, respectively. The pH 8 culture extract of *M. indohii* (-) exhibited a maximum absorbance at 269 nm. Schimek *et al.* (2003) reported that the culture extracts of *Mortierella indohii* showed distinct absorbance spectra for trisporoids with maxima at 291 nm for pH 8 extract and at 304 nm and 283 nm for pH 2 extract. In the interspecific combinations of *M. minutissima* and *P. parasitica*, the spectra of trisporoids could be detected. The most distinct trisporoid spectra were found in the pH 8 combined culture extract of *M. minutissima* (+) and *P. parasitica* (-) as well as in the pH 2 combined culture extract of *M. minutissima* (-) with either (+) or (-) strains of *P. parasitica*. These spectra exhibited maximum absorbance values at 274 and 273 nm, respectively. In this respect, the combined culture extract of (+) and (-) mating types of *M. minutissima* also exhibited distinct absorbance spectra for trisporoids with a single maximum at 272 nm. On the other hand, some *Mortierella* extracts exhibited dark bands on the thin layer chromatography. The darkest tlc bands were recorded in the pH 2 single culture extracts of both *M. globulifera* (+) and *M. minutissima* (+) as well as in the pH 8 combined culture extract of (+) and (-) *M. gamsii*. The pH 8 culture extract of *M. indohii* (+) showed two dark bands for trisporoids that were not found in the pH 2 extract of the same fungus (Schimek *et al*. 2003).

Among the heterothallic species analysed, *Mucor mucedo* (-), *M. racemosus* (+), *Phycomyces blakesleeanus*, and *Thamnidium elegans* were more active for the production of trisporoids than the other strains of Mucorales and Mortierellales, except for *B. trispora* which produced the highest amounts of trisporoids. *M. mucedo* (-) was more active for the production of trisporoids than *M. mucedo* (+). The (-) culture of *M. mucedo* produced relatively high amount of these substances in both pH 2 and pH 8 extracts measured only at 285 nm. Both pH 2 and pH 8 culture extracts of *M. mucedo* (-) exhibited the absorbance spectrum of 4-dihydromethyl trisporate with maxima at 272, 282 nm and a shoulder at 293 nm. Plus and minus strains of *M. mucedo*, as well as in different other mucoralean fungi e.g *B. trispora* produce mating type specific trisporic acid precursors (van den Ende *et al.* 1972) and 4-dihydromethyl trisporate in the culture extract of *M. mucedo* exhibited maximum absorbance at 282 nm (Nieuwenhuis and van den Ende 1975). On the other hand, *M. racemosus* (+) was more active for the production of trisporoids than *M. racemosus* (-) and produced the highest amount of trisporoids in the pH 2 extract, whereas the (+) or (-) cultures of *Phycomyces blakesleeanus*

produced the highest amounts of trisporoids in both pH 2 and pH 8 extracts measured only at 285 nm. *P. blakesleeanus* (-) was more active than *P. blakesleeanus* (+) in this respect. In mated cultures of *B. trispora*, trisporic acid B and C are the major components of trisporoids (Austin *et al*. 1969) but in *P. blakesleeanus*, trisporic acid E is one of the major trisporoids (Miller and Sutter 1984). The analysis of single bands developed on the thin layer chromatography for the culture extracts of *P. blakesleeanus* exhibited the absorbance spectrum of 4-dihydromethyl trisporate with triple maxima at 272, 282 and 294 nm. In both (+) and (-) strains of *Thamnidium elegans*, the highest amounts of trisporoids were recorded in the pH 2 extract and measured only at 285 nm. *T. elegans* (+) was more active than *T. elegans* (-) in this respect. The analysis of culture extacts of these fungi exhibited absorbance spectra similar to those for trisporic acid precursors (Sutter 1970). The indication for the presence of trisporic acid itself, in high concentration, in the mated culture extract of *T. elegans* was not found.

The analysis of culture extracts of these fungi showed distinct absorbance spectra for trisporoids represented in the presence of 4-dihydromethyl trisporate spectrum in several culture extracts of these fungi. Such spectra exhibited a maximum absorbance at 272, 282 nm and a shoulder at 293 nm.

Among the homothallic species analysed, *Gongronella butleri* and *Halteromyces radiatus* were more active for the production of trisporoids than the other species of Mucorales. The highest amounts of these substances were detected in both pH 2 and pH 8 extracts of *G. butleri* measured only at 285 nm, wheras in *H. radiatus* the highest amounts were determined in both pH 2 and pH 8 extracts measured at 285 nm and in the pH 8 extract measured at 325 nm. Although the culture extracts of some species of Mucorales showed relatively low concentration of trisporoids, expressed in the low absorbance values of these extracts, distinct spectra for 4-dihydromethyl trisporate were detected in the spectrophotometric analysis of these extracts. These extracts belonged to *Chaetocladium brefeldii* and *Pilaira anomala*.

The interspecific combination of *Parasitella parasitica* and different zygomycetes obviously stimulated the production of trisporoids in some cases. The induction of trisporoid production was observed in the pH 2 extract of *A. glauca* and *P. parasitica* combinations. The

highest level of trisporoid induction was recorded in the pH 2 combined culture extract of *C. brefeldii* and *P. parasitica* (+) as well as in the pH 2 extract of *L. macrospora* with either (+) or (-) strains of *P. parasitica*. Although trisporoids were highly induced in the pH 2 combination extracts of either *C. brefeldii* or *L. macrospora* with *P. parasitica*, there are main differences between the two fungi. *C. brefeldii* produce low amount of trisporoids in the single culture, whereas high concentration of trisporoids was detected in *L. macrospora* single culture extract. The induction of trisporoids was detected only in the combined culture extracts of *C. brefeldii* and *P. parasitica* (+), but not with *P. parasitica* (-), while in *L. macrospora* high concentration of trisporoids was recorded in the combined culture extracts of *L. macrospora* with either (+) or (-) strains of *P. parasitica*. The most important topic in this respect, that the criteria of taxonomy refer to that the *C. brefeldii* is far away to those of *L. macrospora*. *C. brefeldii* belong to order Mucorales but *L. macrospora* relate to Kickxellales. The production of trisporoids was also induced in the interspecific combination of *P. anomala* and *P. parasitica*. High amount of trisporoids was detected in both pH 2 and pH 8 combined culture extracts of *P. anomala* with either (+) or (-) strains of *P. parasitica*. On the other hand, the analysis of single bands isolated from the thin layer chromatography for the pH 2 combined culture extracts of *P. anomala* and *P. parasitica* exhibited the spectrum of trisporin / trisporol compounds with a maximum at 303 nm. *P. anomala* single extract was not active in this respect and produced only low amounts of trisporoids.

The production of trisporoids also stimulated in the combined cultures of *P. parasitica* and other zygomycetes, but the induction levels were lower compared to those detected in the strains described before. It is worth to mention, that trisporoid production was also reduced in several combinations between *P. parasitica* and different zygomycetes. *B. trispora* produced high amounts of trisporoids only in the combined cultures of (+) and (-) mating types and in the single culture of *B. trispora* (+), but not in combination of cultures with *P. parasitica*. The combined cultures of *B. trispora* (+) and *Z. moelleri* exhibited low concentration of trisporic acid (van den Ende *et al.* 1970).

The first bioassay for trisporoid compounds that stimulated the development of zygophores in *Mucor mucedo* was developed by Plempel (1963 b). Zygophore development

was proportional to the amount of trisporoid tested. Some trisporoids had zygophore-stimulating activities on cultures of *Phycomyces* and *M. mucedo*. In bioassays with both species, 4 dihydromethyl trisporates were active only on (-) cultures. Methyltrisporates are about 100 times more active on (-) cultures than on (+) cultures. Trisporic acids are equally active on both (+) and (-) cultures. Trisporins and trisporols stimulate the development of zygophores in (+), but not in (-) cultures of *M. mucedo* (Bu'Lock *et al.* 1972, 1974 a; Nieuwenhuis and van den Ende 1975). Trisporoids not only initiate the development of zygophores in *M. mucedo*, but also are necessary to maintain them (Gooday 1978; Wurtz and Jockusch 1978).

Among several culture extracts bioassayed for the induction of zygophores in both (+) and (-) strains of *Mucor mucedo*, the mated culture extracts of *Blakeslea trispora* were the most active in the stimulation of zygophores in both (+) and (-) mating types of *M. mucedo*. Similar reaction was also observed in the single culture extracts of *B. trispora*, but the (+) culture extracts were more active than the (-) culture in this respect. Generally, *M. mucedo* (-) was more sensitive for the induction of zygophores than the (+) strain culture, so *B. trispora* extracts exhibited higher numbers of zygophores in *M. mucedo* (-) than in the (+) strain. van den Ende *et al.* (1972) reported that, trisporic acid synthesis in the acid fraction of mated cultures of *B. trispora* was demonstrated by the inducing of zygophores in both mating types of *M. mucedo*. Mating type specific precursors of *B. trispora* was also assayed by using *M. mucedo*. The (+) culture extract of *B. trispora* was active in the stimulation of zygophores in the (-) culture of *M. mucedo* (van den Ende *et al.* 1972; Sutter *et al.* 1973).

The combined culture extracts of *B. trispora* and *P. parasitica* also induced the production of zygospores in *M. mucedo*. The highest numbers of zygophores were detected in *M. mucedo* (-) for the complementary mating types of *B. trispora* and *P. parasitica*. The combined culture extracts of *P. parasitica* (+) and *B. trispora* (-) induced average numbers of 126.5 and 134.75 zygophores in *M. mucedo* (-) in pH 2 and pH 8 extracts, respectively, whereas average numbers of 61.5 and 93.5 were recorded in the combined culture extracts of *P. parasitica* (-) and *B. trispora* (+) at pH 2 and pH 8, respectively. Production of trisporic acid in the host-parasite system could be observed in the *A. glauca-P. parasitica* interaction (Wöstemeyer *et al.* 1997 a).

Absidia glauca culture extracts induced the production of zygophores only in *Mucor mucedo* (-). No zygophores were detected in *M. mucedo* (+) for all culture extracts of *A. glauca*. The single culture extracts of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ were more active for the induction of zygophores in *M. mucedo* (-) than *A. glauca*$_{6776a}$ and *A. glauca*$_{6776b}$ as well as in the combined culture extracts of mated *A. glauca* and in combinations with *P. parasitica*. The highest numbers of zygophores were detected in *M. mucedo* (-) for the complementary mating types of *A. glauca* and *P. parasitica* as well as in *B. trispora* extracts. The combined culture extracts of *P. parasitica* (+) and *A. glauca*$_{101.48}$ (-) induced average numbers of 92.5 and 68.25 zygophores in pH 2 and pH 8 extracts, respectively, whereas averages of 124.5 and 112 zygophores were recorded in the combined culture extracts of *P. parasitica* (-) and *A. glauca*$_{100.48}$ (+) at pH 2 and pH 8 extracts, respectively. Compared to the numbers of zygophores recorded in the single culture extracts of *A. glauca*$_{100.48}$ (+) and *A. glauca*$_{101.48}$ (-), the combined culture extracts of the complementary mating types of *A. glauca* and *P. parasitica* were more active in this respect. The mated culture extracts of *A. glauca*$_{100.48}$ and *A. glauca*$_{101.48}$ also induced zygophore production in *M. mucedo* (-) and the induction effect was higher in pH 2 extract (116 zygophores) than in the pH 8 (54.75). Wöstemeyer *et al.* (1995) reported that, trisporic acid is formed by a cooperative biosynthesis involving both mating types and also seems to be involved in mediating the recognition between *P. parasitica* and *A. glauca*. For more evidence in the direct hormone correlations between sex and parasitism, one of the key enzymes for trisporic acid synthesis, 4-dihydromethyl trisporate dehydrogenase, has been isolated and sequenced and the corresponding gene has been identified and cloned in *M. mucedo* (Czempinski *et al.* 1996).

This work not only conducted in the ability of *Parasitella parasitica* to infect several members of mucoralean fungi but also aimed to investigate such reaction with other members of zygomycetes such as Mortierellales and Kickxellales.

Parasitella parasitica is a facultative mycoparasite of many mucoralean fungi. The parasitic interaction usually occures between the aerial hyphae of the parasite and its hosts. The infection process includes the formation of a plasmatic continuum between the parasite and its

host, which allow the invasion of the host by nuclei of the parasite. Then the host forms a 'gall' around the infection site. In case of a complete parasitic development the infection structure developes to form a sikyospore.

The parasitic interaction of *A. glauca* and *P. parasitica* is mating type specific reaction. Strong parasitic interaction occured between *P. parasitica* (+) and *A. glauca* (-). Such a reaction was not found in the identical mating types of *P. parasitica* and *A. glauca*. Burgeff (1924) analysed the sexual and parasitic interactions in *Mucor*-like fungi at the microscopic level. One of the most important observations was, that the chemical principle inducing sexual development in these fungi seemed to be universal within the group. Burgeff also described the interaction between the facultative mycoparasite *P. parasitica* and different hosts, including *A. glauca*. The most important observation in that respect was the dissolving of the contact zone of cell walls of both partners and transfer of genetic material from the parasite into the host mycelium. Recently, this kind of mycoparasitism was classified as fusion biotrophs (Jeffries and Young 1994). The host-parasite system *A. glauca-P. parasitica* was extensively studied as well as the transfer of genetic material from *P. parasitica* into the mycelium of *A. glauca* (Kellner *et al.* 1993; Wöstemeyer *et al.* 1995, 1997 a, b).

Parasitella parasitica also exclusively infected several members of homothallic and heterothallic mucoralean fungi including *Absidia parricida* (Absidiaceae), *Blakeslea trispora* (Choanephoraceae), *Chaetocladium brefeldii* (Chaetocladiaceae), *Gilbertella persicaria* (Gilbertellaceae), *Mucor mucedo* and *M. racemosus* (Mucoraceae), *Mycotypha africana* (Mycotyphaceae), *Thamnidium elegans* (Thamnidiaceae) and *Zygorhynchus moelleri* (Mucoraceae). Similar reaction was previously observed in several members of the Mucorales including *Mucor mucedo, M. racemosus*, *Thamnidium elegans* and *Zygorhynchus exponens* (Burgeff 1924, table 4). Parasitic interactions were not found in different heterothallic *Mortierella* species including *M. gamsii*, *M. globulifera*, *M. humilis*, *M. indohii*, *M. minutissima* and *M. parvispora*. Burgeff (1924) also reported no reaction between *P. simplex* and *Mortierella* sp.

The strength of the parasitic interaction was variable with different hosts. In some heterothallic species including *B. trispora* (+), *B. trispora* (-), *M. mucedo* (-), *M. racemosus*

(+), *M. racemosus* (-) and *T. elegans* (+), strong parasitic interaction with the formation of typical sikyospores was detected with either (+) or (-) strains of *P. parasitica*. Such reaction was not observed in case of *M. mucedo* (+) that showed only little interaction with *P. parasitica* (+). In this respect, *P. parasitica* (+) also showed strong parasitic interaction with the formation of several sikyospores with *T. elegans* (-) whereas little interaction was observed for *P. parasitica* (-) with the (+) host strain of *T. elegans*.

The homothallic *A. parricida*, *G. persicaria*, *M. africana* and *Z. moelleri* showed strong parasitic interaction with the formation of several sikyospores with either (+) or (-) *P. parasitica*, whereas *C. brefeldii* showed strong parasitic interaction only with *P. parasitica* (+).

Other systems of parasitism are found in the nature, including the specialized conjugative system between *Agrobacterium tumefaciens* and its host plants, which results in the transfer of the Ti plasmid into the plant genome (Weising *et al.* 1988). Moreover, the parasitism of the marine red alga *Choreocolax* on its host red alga *Polysiphonia* involves the transfer of planetic nuclei via plasma bridges (Goff and Coleman 1984).

Zygospore formation in members of homothallic and heterothallic zygomycetes was observed on the solidified supplemented minimal medium. Zygospore-forming species were belonged to many genera including *Absidia*, *Blakeslea*, *Mortierella*, *Mucor*, *Parasitella*, *Thamnidium* and *Zygorhynchus*. The formation of zygospores under the conditions employed was detected in three heterothallic species of *Mortierella*, including M. *humilis*, *M. indohii* and *M. parvispora*, whereas *M. gamsii*, *M. globulifera* and *M. minutissima* did not produce zygospores under these conditions. The sexual reaction and production of zygospores in several members of mucoralean fungi were thoroughly studied (Burgeff 1924; Blakeslee and Cartledge 1927; Blakeslee *et al.* 1927; Gooday 1973; Schipper 1975, 1976, 1978; Schipper *et al.* 1975; Havens 1976; Stalpers and Schipper 1980; Sutter 1987; Mathur and Sarbhoy 1999) as well as in homothallic and heterothallic *Mortierella* species (Gams *et al.* 1972; Chien *et al*. 1974; Ansell and Young 1983; Degawa and Tokumasu 1998 a, b; Schimek *et al.* 2003).

Table 4: Extent of parasitic development between *P. simplex* and different zygomycetes as reported by Burgeff (1924).

Host	Sex of host	*P. simplex* (+)	*P. simplex* (-)
Absidia glauca Lendner	(+)	--	GF
A. glauca „	(-)	GF	--
A. orchidis „	(+)	--	SK
A. orchidis „	(-)	GF	--
A. cylindrospora Hagem	(+)	--	--
A. cylindrospora „	(-)	--	--
A. spinosa Lendner	(±)	SK*	SK*
Chaetocladium brefeldi van Tiegh.& Lemonnier var. *macrosporum* Burgeff	(+)	--	--
Chaetostylum Fresenii van Tiegh.& Lemonnier	?	SK&GF	SK&GF
Choanephora curcurbitarum Thaxter	?	GF	GF
Cunninghamella elegans Lendner	?	--	--
Helieostylum piriforme Bainier	?	--	--
Mucor mucedo L.	(+)	SK	SK
M. mucedo L.	(-)	SK	SK
M. mucilagineus Brefeld	?	SK	SK
M. genereusis Lendner	?	GF	GF
M. dubius Wehmer	?	G	G
M. spinosus van Tiegh.	?	GF	GF
M. bamannianus Möller	?	--	--
M. hiemalis Hagem	(+)	GF	GF
M. hiemalis „	(-)	GF	GF
M. racemosus Fres.	?	SK	SK
M. tenuis Bainier	(±)	SK	SK
Mortierella sp.	(±)	--	--
Parasitella simplex Bainier	(+)	--	Zygs.
P. simplex „	(-)	Zygs.	--
Phycomyces nitens Kunze	(+)	--	--
P. nitens „	(-)	--	--
Phycomyces sp.	(+)	--	--
Phycomyces sp.	(-)	--	--
Pilaira anomala Schroeter	?	GF	GF
Pilobolus sp.	?	--	--
Rhizopus nigricans Ehrenberg	(+)	G	G
R. nigricans „	(-)	GF	GF
R. nigricans „ Nr. 7	'neutr.'	GF	GF

Table 4: Continued

Host	Sex of host	*P. simplex* (+)	*P. simplex* (-)
Sporodinia grandis Link	(±)	GF**	GF**
Syncephalastrum racemosum Schroeter	?	--	--
S. cinereum	?	GF	--
Thamnidium elegans Link	?	GF	GF
T. elegans „	?	SK	SK
Zygorhynchus exponens Burgeff	(±)	GF	GF

GF = Gall formation, SK = Sikyospore formation, Zygs = Zygospore formation, -- = No interaction
** = Infection only at the sexual organs of the host

5. SUMMARY

1. All zygomycetes analysed have the ability to produce trisporoids, but at various concentration levels as it is expressed by the spectrophotometric measurements and thin layer chromatography of their culture extracts.

2. Among thirty species of homothallic and heterothallic zygomycetes, *Blakeslea trispora* was the most active for the production of trisporoids. *B. trispora* (+) produced very high amounts of trisporic acid precursors that exhibited maximum absorbance at 283 and 284 nm for pH 2 and pH 8 culture extracts, respectively. In the pH 2 mated culture extract of *B. trispora* very high concentration of trisporic acid was detected. This compound exhibited an absorbance maximum at 322 nm. Trisporic acid precursors in the pH 8 mated culture extract of *B. trispora* exhibited maximum absorbance at 232 and 288 nm.

3. Presence of trisporic acid compounds in the culture extracts of *Blakeslea trispora* separated into several dark bands on the analytical thin layer chromatography.

4. Production of trisporoids varied among different species of homothallic and heterothallic Mucorales as well as between members of Mortierellales and Kickxellales.

5. *Coemansia formosensis* and *Linderina macrospora* produced high amounts of trisporoids. The high amounts of these substances were measured at 285 nm for *C. formosensis* and exhibited maximum absorbance at 271 nm. *L. macrospora* is more active than *C. formosensis* in this respect. The highest amounts of trisporoids produced by *L. macrospora* were detected in the pH 2 culture extract and exhibited maximum absorbance at 278 nm.

6. Production of trisporoids, in high concentrations, in the culture extracts of *C. formosensis* as well as in the pH 2 culture extract of *L. macrospora* separated into dark bands on the thin layer chromatography.

7. In the host-parasite system, as in the interspecific combinations of *Parasitella parasitica* and different zygomycetes, the production of trisporoids was stimulated in case of *Absidia glauca, Chaetocladium brefeldii, Linderina macrospora* and *Pilaira anomala*.

8. Production of trisporoids was highly increased in the pH 2 combined culture extract of *P. parasitica* and *A. glauca* as well as in the homothallic *C. brefeldii, L. macrospora* and *P. anomala*.

9. The high amount of trisporoids in the pH 2 combined culture extract of *P. parasitica* (+) and *C. brefeldii* exhibited maximum absorbance at 312 nm, while the pH 8 culture extract of the

same combination showed the absorbance spectrum of 4-dihydromethyl trisporate with triple maxima at 272, 282 nm and a shoulder at 293 nm.

10. The three-components spectrum of 4-dihydromethyl trisporate was common among several culture extracts analysed. The 4-dihydromethyl trisporate spectrum was detected in several culture extracts of Mucoralean fungi including *Chaetocladium brefeldii*, *Cunninghamella elegans*, *Gilbertella persicaria*, *Gongronella butleri*, *Halteromyces radiatus*, *Mucor mucedo*, *M. racemosus*, *Phycomyces blakesleeanus*, *Syzigites megalocarpus* and *Thamnidium elegans* as well as in the combined culture extracts of these fungi and *P. parasitica*.

11. The spectrophotometric analysis of single band isolated from the thin layer chromatography for the combined culture extract of (+) and (-) mating types of *P. parasitica* exhibited the spectrum of 4- dihydromethyl trisporate with triple maxima at 272, 282 and 294 nm, whereas the bands isolated from the single culture extract of *P. parasitica* (-) exhibited the absorbance spectrum of trisporin / trisporol compounds with a single maxima at 301 and 302 nm for pH 2 and pH 8 extracts, respectively.

12. *Parasitella parasitica* is not only the facultative mycoparasite of mucoralean fungi, but also able to infect fungi related to other orders of zygomycetes such as the Kickxellales species, *Linderina macrospora*.

13. The parasitic interaction in *P. parasitica-A. glauca* system is strongly mating type specific reaction. *P. parasitica* infect only the complementary mating types of *A. glauca*. No interaction was observed between the identical mating types of these fungi. The highest level of parasitic interaction was detected between *P. parasitica* (+) and *A. glauca* (-).

14. *P. parasitica* infected several members of homothallic and heterothallic species of zygomycetes, but the strength of the infection was varied between different strains tested.

15. Sikyospore formation was detected in several combinations of *P. parasitica* and different hosts. These hosts are members of zygomycetes.

16. Sexual interaction and zygospore formation were observed in the combined cultures of (+) and (-) mating types of several heterothallic species, under the conditions employed in this work, including *Absidia*, *Blakeslea*, *Mortierella*, *Mucor*, *Parasitella* and *Thamnidium*.

Zygospore formation in the homothallic zygomycetes species could be detected under these conditions, especially in *Absidia parricida* and *Zygorhynchus moelleri*.

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7. List of Abbreviations

Abbreviation	Explanation	Abbreviation	Explanation
SDS	Sodium dodecyl sulphate	C.b	*Chaetocladium brefeldii*
PAGE	Polyacrylamide gel electrophoresis	C.f	*Coemansia formosensis*
PVDF	Polyvinylidene difluoride	C.e	*Cunninghamella elegans*
PCR	Polymerase chain reaction	G.p	*Gilbertella persicaria*
DNA	Deoxy ribonucleic acid	G.b	*Gongronella butleri*
FSU	Friedrich Schiller University, Jena,	H.r	*Halteromyces radiatus*
	Germany.	L.m	*Linderina macrospora*
CBS	Centraalbureau voor	M.ga	*Mortierella gamsii*
	Schimmelcultures, Netherlands.	M.gl	*Mortierella globulifera*
ATCC	American Type Culture Collection.	M.h	*Mortierella humilis*
P	Phycomycetes, Fungal Reference	M.i	*Mortierella indohii*
	Center, Jena, Germany.	M.m	*Mortierella minutissima*
DSM	Deutsche Stammsammlung von	M.p	*Mortierella parvispora*
	Mikroorganismen, Germany.	M.mu	*Mucor mucedo*
NRRL	Norther Regional Research	M.r	*Mucor racemosus*
	Laboratories, America.	M.a	*Mycotypha africana*
O	Order	P.b	*Phycomyces blakesleeanus*
F	Family	P.a	*Pilaira anomala*
tlc	Thin layer chromatography	S.r	*Syncephalastrum racemosum*
UV	Ultra violet	S.m	*Syzigites megalocarpus*
Fig.	Figure	T.e	*Thamnidium elegans*
Conc	Concentration	T.p	*Thamnostylum piriforme*
TSA	Trisporic acid	Z.m	*Zygorhynchus moelleri*
P.p	*Parasitella parasitica*	S.i	Strong interaction
A.g	*Absidia glauca*	L.i	Little interaction
A.o	*Absidia orchidis*	Sk	Sikyospore
A.p	*Absidia parricida*		
A.s	*Absidia spinosa*		
A.e	*Actinomucor elegans*		
B.t	*Blakeslea trispora*		

Table 5: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and *Absidia glauca.* P.p = *P. parasitica*, A.g = *A. glauca*

Strains	Absorbance				Conc. of(µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
A. glauca 100.48 (+)	10.63	16.83	6.15	15.95	0.194	0.308	0.108	0.279
A. glauca 101.48 (-)	9.94	12.81	3.86	6.24	0.182	0.234	0.067	0.109
A. glauca 6776a (+)	18.67	11.67	5.46	6.71	0.341	0.213	0.095	0.117
A. glauca 6776b (-)	26.23	24.82	18.56	33.42	0.480	0.454	0.324	0.584
P.p (+) x P.p (-)	25.09	17.85	9.65	8.09	0.459	0.326	0.169	0.141
P.p (+) x A.g 100.48 (+)	43.95	15.14	19.22	9.27	0.803	0.277	0.336	0.162
P.p (+) x A.g 101.48 (-)	43.29	16.07	16.77	5.01	0.791	0.294	0.293	0.088
P.p (-) x A.g 100.48 (+)	35.61	19.42	16.34	15.23	0.651	0.355	0.286	0.266
P.p (-) x A.g 101.48 (-)	31.11	23.56	12.28	8.76	0.569	0.431	0.215	0.153
A.g 100.48 (+) x A.g 101.48 (-)	37.53	17.19	17.42	16.08	0.686	0.314	0.305	0.281
P.p (+) x P.p (-)	9.75	7.21	4.64	2.70	0.178	0.132	0.081	0.047
P.p (+) x A.g 6776a (+)	5.84	6.44	3.14	2.66	0.107	0.118	0.055	0.047
P.p (+) x A.g 6776b (-)	9.56	5.25	4.04	3.16	0.175	0.096	0.071	0.055
P.p (-) x A.g 6776a (+)	6.87	4.53	3.61	2.50	0.126	0.083	0.063	0.044
P.p (-) x A.g 6776b (-)	6.71	5.82	3.64	4.95	0.123	0.106	0.064	0.087
A.g 6776a (+) x A.g 6776b (-)	8.38	4.65	5.24	4.41	0.153	0.085	0.092	0.077

Table 6: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different species of *Absidia.* P.p = *P. parasitica*, A.o = *A. orchidis*, A.p = *A. parricida*, A.s = *A. spinosa*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
A. orchidis	21.33	8.37	9.43	8.72	0.390	0.153	0.165	0.152
A. parricida	20.60	9.98	9.38	12.34	0.377	0.182	0.164	0.216
A. spinosa	13.99	10.18	4.99	0.86	0.256	0.186	0.087	0.015
P.p (+) x P.p (-)	18.40	5.78	5.80	2.15	0.336	0.106	0.101	0.038
P.p (+) x A.o	22.05	8.40	7.78	4.78	0.403	0.154	0.136	0.084
P.p (-) x A.o	20.03	7.65	8.20	6.27	0.366	0.140	0.143	0.110
P.p (+) x P.p (-)	23.00	7.46	11.35	1.61	0.420	0.136	0.198	0.028
P.p (+) x A.p	13.99	6.62	5.08	3.95	0.256	0.121	0.089	0.069
P.p (-) x A.p	10.11	7.76	4.23	6.30	0.185	0.142	0.074	0.110
P.p (+) x P.p (-)	11.11	14.18	3.07	2.99	0.203	0.259	0.054	0.052
P.p (+) x A.s	16.94	21.43	4.40	4.47	0.310	0.392	0.077	0.078
P.p (-) x A.s	11.20	6.62	3.45	0.94	0.205	0.121	0.060	0.016

Table 7: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, A.e = *A. elegans*, B.t = *B. trispora*, C.b = *C. brefeldii*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
A. elegans	16.11	11.65	7.01	3.52	0.295	0.213	0.123	0.062
B. trispora (+)	74.36	289.5	30.30	80.10	1.359	5.293	0.530	1.400
B. trispora (-)	13.45	10.18	4.26	3.87	0.246	0.186	0.074	0.068
C. brefeldii	14.63	4.98	3.15	1.07	0.267	0.091	0.055	0.019
P.p (+) x P.p (-)	10.92	7.06	4.57	3.30	0.200	0.129	0.080	0.058
P.p (+) x A.e	17.47	15.31	6.56	4.52	0.319	0.280	0.115	0.079
P.p (-) x A.e	18.13	12.55	7.07	3.32	0.331	0.229	0.124	0.058
P.p (+) x P.p (-)	15.56	11.03	9.07	6.27	0.284	0.202	0.159	0.110
P.p (+) x B.t (+)	15.14	16.15	6.72	7.26	0.277	0.295	0.117	0.127
P.p (+) x B.t (-)	25.68	13.03	9.41	6.10	0.469	0.238	0.165	0.107
P.p (-) x B.t (+)	22.90	24.28	12.28	10.24	0.419	0.444	0.215	0.179
P.p (-) x B.t (-)	13.00	15.30	6.75	8.01	0.238	0.280	0.118	0.140
B.t (+) x B.t (-)	166.0	249.2	236.8	131.4	3.035	4.556	4.140	2.297
P.p (+) x P.p (-)	7.45	8.98	1.55	1.95	0.136	0.164	0.027	0.034
P.p (+) x C.b	151.7	39.40	164.5	15.05	2.773	0.720	2.876	0.263
P.p (-) x C.b	18.52	16.38	7.78	4.47	0.339	0.299	0.136	0.078

Table 8: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, C.f = *C. formosensis*, C.e = *C. elegans*, G.p = *G. persicaria*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
C. formosensis	45.88	69.86	5.94	3.90	0.839	1.277	0.104	0.068
C. elegans	20.01	14.38	6.96	2.76	0.366	0.263	0.122	0.048
G. persicaria	22.05	13.65	7.31	3.09	0.403	0.250	0.128	0.054
P.p (+) x P.p (-)	25.83	15.85	5.35	4.35	0.472	0.290	0.094	0.076
P.p (+) x C.f	37.93	21.64	8.27	4.50	0.693	0.396	0.145	0.079
P.p (-) x C.f	22.97	11.41	6.35	3.17	0.420	0.209	0.111	0.055
P.p (+) x P.p (-)	18.40	5.78	5.80	2.15	0.336	0.106	0.101	0.038
P.p (+) x C.e	11.13	12.01	3.34	3.00	0.203	0.220	0.058	0.052
P.p (-) x C.e	22.18	10.16	7.30	3.81	0.405	0.186	0.128	0.067
P.p (+) x P.p (-)	25.83	15.85	5.35	4.35	0.472	0.290	0.094	0.076
P.p (+) x G.p	36.58	23.29	11.75	5.55	0.669	0.426	0.205	0.097
P.p (-) x G.p	32.99	14.31	10.73	4.08	0.603	0.262	0.188	0.071

Table 9: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, G.b = G. butleri, H.r = H. radiatus, L.m = L. macrospora.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
G. butleri	28.70	16.93	5.02	2.89	0.525	0.310	0.088	0.051
H. radiatus	25.06	24.37	7.76	7.02	0.458	0.446	0.136	0.123
L. macrospora	129.8	46.27	90.5	22.30	2.373	0.846	1.582	0.390
P.p (+) x P.p (-)	25.83	15.85	5.35	4.35	0.472	0.290	0.094	0.076
P.p (+) x G.b	28.12	21.86	5.91	5.66	0.514	0.400	0.103	0.099
P.p (-) x G.b	31.78	17.04	6.21	4.04	0.581	0.312	0.109	0.071
P.p (+) x P.p (-)	25.83	15.85	5.35	4.35	0.472	0.290	0.094	0.076
P.p (+) x H.r	42.80	21.67	12.54	5.39	0.782	0.396	0.219	0.094
P.p (-) x H.r	23.38	28.04	7.33	8.57	0.427	0.513	0.128	0.150
P.p (+) x P.p (-)	23.00	7.46	11.35	1.61	0.420	0.136	0.198	0.028
P.p (+) x L.m	156.9	34.35	154.8	21.44	2.868	0.628	2.706	0.375
P.p (-) x L.m	126.8	31.81	123.9	17.06	2.318	0.582	2.166	0.298

Table 10: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different *Mortierella* species. P.p = *P. parasitica*, M.ga = *M. gamsii*, M.gl = *M. globulifera*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
M. gamsii (+)	6.29	5.36	1.32	1.94	0.115	0.098	0.023	0.034
M. gamsii (-)	10.23	5.82	3.81	1.37	0.187	0.106	0.067	0.024
M. globulifera (+)	8.25	5.28	2.77	0.98	0.151	0.097	0.048	0.017
M. globulifera(-)	15.49	8.39	8.29	2.27	0.283	0.153	0.145	0.040
P.p (+) x P.p (-)	33.67	22.31	13.94	6.34	0.616	0.408	0.244	0.111
P.p (+) x M.ga (+)	27.91	16.19	11.88	4.63	0.510	0.296	0.208	0.081
P.p (+) x M.ga (-)	21.11	17.87	9.12	5.30	0.386	0.327	0.159	0.093
P.p (-) x M.ga (+)	24.76	18.77	10.81	5.63	0.453	0.343	0.189	0.098
P.p (-) x M.ga (-)	29.71	13.05	12.28	3.71	0.543	0.239	0.215	0.065
M.ga (+) x M.ga (-)	32.79	14.78	13.06	4.28	0.599	0.270	0.228	0.075
P.p (+) x P.p (-)	13.85	8.22	4.09	2.52	0.253	0.150	0.072	0.044
P.p (+) x M.gl (+)	29.41	41.87	12.90	22.43	0.538	0.765	0.226	0.392
P.p (+) x M.gl (-)	10.36	11.19	3.70	3.71	0.189	0.205	0.065	0.065
P.p (-) x M.gl (+)	14.77	21.77	5.63	11.72	0.270	0.398	0.098	0.205
P.p (-) x M.gl (-)	13.20	8.85	5.75	3.21	0.241	0.162	0.101	0.056
M.gl (+) x M.gl (-)	17.14	10.58	7.06	3.62	0.313	0.193	0.123	0.063

Table 11: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different *Mortierella* species. P.p = *P. parasitica*, M.h = *M. humilis*, M.i = *M. indohii*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
M. humilis (+)	10.40	9.52	6.03	3.99	0.190	0.174	0.105	0.070
M. humilis (-)	11.97	8.13	5.16	3.85	0.219	0.149	0.090	0.067
M. indohii (+)	13.74	5.13	8.00	3.02	0.251	0.094	0.140	0.053
M. indohii (-)	13.49	8.61	7.47	3.59	0.247	0.157	0.131	0.063
P.p (+) x P.p (-)	15.07	11.89	4.76	3.32	0.276	0.217	0.083	0.058
P.p (+) x M.h (+)	8.15	6.00	2.43	2.26	0.149	0.110	0.042	0.040
P.p (+) x M.h (-)	8.62	8.92	2.99	2.90	0.158	0.163	0.052	0.051
P.p (-) x M.h (+)	7.49	7.43	3.65	2.52	0.137	0.136	0.064	0.044
P.p (-) x M.h (-)	11.13	7.68	3.69	1.95	0.203	0.140	0.065	0.034
M.h (+) x M.h (-)	8.12	12.80	2.74	4.72	0.148	0.234	0.048	0.083
P.p (+) x P.p (-)	28.37	9.69	8.45	1.24	0.519	0.177	0.148	0.022
P.p (+) x M.i (+)	13.31	7.30	4.51	0.99	0.243	0.133	0.079	0.017
P.p (+) x M.i (-)	14.53	9.75	5.17	2.35	0.266	0.178	0.090	0.041
P.p (-) x M.i (+)	24.06	6.42	13.93	2.05	0.440	0.117	0.244	0.036
P.p (-) x M.i (-)	11.97	3.78	5.61	0.40	0.219	0.069	0.098	0.007
M.i (+) x M.i (-)	23.02	5.77	8.47	1.54	0.421	0.105	0.148	0.027

Table 12: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different *Mortierella* species. P.p = *P. parasitica*, M.m = *M. minutissima*, M.p = *M. parvispora*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
M. minutissima (+)	13.45	5.47	6.07	2.56	0.246	0.100	0.106	0.045
M. minutissima (-)	14.94	11.88	6.40	4.75	0.273	0.217	0.112	0.083
M. parvispora (+)	7.76	4.24	0.98	1.41	0.142	0.078	0.017	0.025
M. parvispora (-)	4.31	3.33	0.81	1.17	0.079	0.061	0.014	0.020
P.p (+) x P.p (-)	15.16	10.83	7.99	5.79	0.277	0.198	0.140	0.101
P.p (+) x M.m (+)	21.36	9.28	9.31	4.79	0.390	0.170	0.163	0.084
P.p (+) x M.m (-)	20.57	9.46	8.29	3.95	0.376	0.173	0.145	0.069
P.p (-) x M.m (+)	13.79	10.19	4.78	2.82	0.252	0.186	0.084	0.049
P.p (-) x M.m (-)	29.82	8.65	9.48	3.49	0.545	0.158	0.166	0.061
M.m (+) x M.m (-)	12.93	9.44	5.11	2.79	0.236	0.173	0.089	0.049
P.p (+) x P.p (-)	18.91	12.38	7.59	6.90	0.346	0.226	0.133	0.121
P.p (+) x M.p (+)	13.07	12.95	6.95	7.02	0.239	0.237	0.122	0.123
P.p (+) x M.p (-)	19.14	9.02	9.19	5.56	0.350	0.165	0.161	0.097
P.p (-) x M.p (+)	13.13	11.81	7.30	5.35	0.240	0.216	0.128	0.094
P.p (-) x M.p (-)	10.83	9.67	0.98	0.06	0.198	0.177	0.017	0.001
M.p (+) x M.p (-)	17.35	13.09	8.58	6.04	0.317	0.239	0.150	0.106

Table 13: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, M.mu = *M. mucedo*, M.r = *M. racemosus*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
M. mucedo (+)	16.16	9.47	6.98	3.83	0.295	0.173	0.122	0.067
M. mucedo (-)	24.49	15.11	8.55	4.17	0.448	0.276	0.149	0.073
M. racemosus (+)	47.38	13.00	15.08	3.62	0.866	0.238	0.264	0.063
M. racemosus (-)	23.78	9.56	8.33	2.28	0.435	0.175	0.146	0.040
P.p (+) x P.p (-)	7.80	11.43	3.29	3.62	0.143	0.209	0.058	0.063
P.p (+) x M.mu (+)	8.30	12.95	3.85	4.33	0.152	0.237	0.067	0.076
P.p (+) x M.mu (-)	9.94	21.88	3.88	6.95	0.182	0.400	0.068	0.122
P.p (-) x M.mu (+)	9.09	6.47	2.80	1.44	0.166	0.118	0.049	0.025
P.p (-) x M.mu (-)	12.27	6.04	5.73	1.84	0.224	0.110	0.100	0.032
M.mu (+) x M.mu (-)	10.68	12.19	4.25	4.36	0.195	0.223	0.074	0.076
P.p (+) x P.p (-)	16.50	11.73	6.60	3.10	0.302	0.214	0.115	0.054
P.p (+) x M.r (+)	24.59	15.78	9.19	3.62	0.450	0.288	0.161	0.063
P.p (+) x M.r (-)	17.17	11.79	7.54	3.17	0.314	0.216	0.132	0.055
P.p (-) x M.r (+)	18.21	19.53	7.54	5.30	0.333	0.357	0.132	0.093
P.p (-) x M.r (-)	27.09	10.16	11.35	2.16	0.495	0.186	0.198	0.038
M.r (+) x M.r (-)	15.30	6.75	6.08	1.15	0.280	0.123	0.106	0.020

Table 14: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, M.a = *M. africana*, P.b = *P. blakesleeanus*,

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
M. africana	21.33	3.86	8.53	0.75	0.390	0.071	0.149	0.013
P. blakesleeanus (+)	33.18	24.63	8.83	4.55	0.607	0.450	0.154	0.080
P. blakesleeanus (-)	42.32	31.36	9.67	5.19	0.774	0.573	0.169	0.091
P.p (+) x P.p (-)	23.00	7.46	11.35	1.61	0.420	0.136	0.198	0.028
P.p (+) x M.a	12.77	5.28	2.88	0.93	0.233	0.097	0.050	0.016
P.p (-) x M.a	6.25	3.75	2.04	0.67	0.114	0.069	0.036	0.012
P.p (+) x P.p (-)	37.34	22.71	5.87	4.86	0.683	0.415	0.103	0.085
P.p (+) x P.b (+)	34.26	24.09	18.10	5.55	0.626	0.440	0.316	0.097
P.p (+) x P.b (-)	32.83	23.42	8.52	4.73	0.600	0.428	0.149	0.083
P.p (-) x P.b (+)	31.51	27.21	9.58	3.78	0.576	0.497	0.167	0.066
P.p (-) x P.b (-)	21.75	21.70	5.57	3.94	0.398	0.397	0.097	0.069
P.b (+) x P.b (-)	40.28	25.96	10.21	4.58	0.736	0.475	0.178	0.080

Table 15: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, P.a = *P. anomala*, S.r = *S. racemosum*, S.m = *S. megalocarpus*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
P. anomala	11.50	9.63	4.70	2.62	0.210	0.176	0.082	0.046
S. racemosum	15.17	7.13	5.88	2.63	0.277	0.130	0.103	0.046
S. megalocarpus	19.30	15.42	6.87	2.69	0.353	0.282	0.120	0.047
P.p (+) x P.p (-)	16.50	11.73	6.60	3.10	0.302	0.214	0.115	0.054
P.p (+) x P.a	41.08	28.24	29.66	7.44	0.751	0.516	0.519	0.130
P.p (-) x P.a	50.42	22.59	47.23	5.91	0.922	0.413	0.826	0.103
P.p (+) x P.p (-)	18.40	5.78	5.80	2.15	0.336	0.106	0.101	0.038
P.p (+) x S.r	12.16	7.19	3.97	2.15	0.222	0.131	0.069	0.038
P.p (-) x S.r	11.06	5.85	4.00	2.35	0.202	0.107	0.070	0.041
P.p (+) x P.p (-)	23.00	7.46	11.35	1.61	0.420	0.136	0.198	0.028
P.p (+) x S.m	12.00	12.65	3.18	1.85	0.219	0.231	0.056	0.032
P.p (-) x S.m	18.51	8.42	5.84	0.98	0.338	0.154	0.102	0.017

Table 16: Production of trisporic acid substances in inter- and intraspecific interactions of *Parasitella parasitica* and different zygomycetes. P.p = *P. parasitica*, T.e = *T. elegans*, T.p = *T. piriforme*, Z.m = *Z. moelleri*.

Strains	Absorbance				Conc. of (µg/ml) precursors		Conc. of TSA (µg/ml)	
	285 nm		325 nm					
	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	39.60	18.97	14.40	5.63	0.724	0.347	0.252	0.098
P. parasitica (-)	10.21	9.73	3.68	2.96	0.187	0.178	0.064	0.052
T. elegans (+)	44.67	14.83	8.36	2.63	0.817	0.271	0.146	0.046
T. elegans (-)	30.34	15.08	7.71	3.79	0.555	0.276	0.135	0.066
T. piriforme	20.12	7.71	8.00	2.60	0.368	0.141	0.140	0.045
Z. moelleri	14.73	10.46	5.11	1.37	0.269	0.191	0.089	0.024
P.p (+) x P.p (-)	7.45	8.98	1.55	1.95	0.136	0.164	0.027	0.034
P.p (+) x T.e (+)	18.23	9.94	4.99	1.76	0.333	0.182	0.087	0.031
P.p (+) x T.e (-)	13.29	14.77	4.28	3.12	0.243	0.270	0.075	0.055
P.p (-) x T.e (+)	24.89	14.58	5.19	2.89	0.455	0.267	0.091	0.051
P.p (-) x T.e (-)	23.11	13.01	5.73	3.33	0.422	0.238	0.100	0.058
T.e (+) x T.e (-)	56.01	15.74	5.54	3.22	1.024	0.288	0.097	0.056
P.p (+) x P.p (-)	25.83	15.85	5.35	4.35	0.472	0.290	0.094	0.076
P.p (+) x T.p	20.44	16.76	5.99	4.45	0.374	0.306	0.105	0.078
P.p (-) x T.p	24.69	26.19	9.63	9.68	0.451	0.479	0.168	0.169
P.p (+) x P.p (-)	11.11	14.18	3.07	2.99	0.203	0.259	0.054	0.052
P.p (+) x Z.m	9.92	10.29	3.00	1.74	0.181	0.188	0.052	0.030
P.p (-) x Z.m	13.30	11.54	4.09	2.59	0.243	0.211	0.072	0.045

Table 17: Effect of fungal extracts on the induction of zygophores in *Mucor mucedo.*
P.p = *P. parasitica*, A.g = *A. glauca*

Fungal extract (10 µg)	Average no. of zygophores of *Mucor mucedo* (+)		Average no. of zygophores of *Mucor mucedo* (-)	
	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	--	--	--	--
P. parasitica (-)	--	--	--	--
A. glauca $_{100.48}$ (+)	--	--	61.5	35.75
A. glauca $_{101.48}$ (-)	--	--	54.25	27.5
A. glauca $_{6776a}$ (+)	--	--	14.5	9.5
A. glauca $_{6776b}$ (-)	--	--	6.75	4.5
P.p (+) x P.p (-)	--	--	0.5	--
P.p (+) x A.g $_{100.48}$ (+)	--	--	89.5	55.75
P.p (+) x A.g $_{101.48}$ (-)	--	--	92.5	68.25
P.p (-) x A.g $_{100.48}$ (+)	--	--	124.5	112
P.p (-) x A.g $_{101.48}$ (-)	--	--	2.75	--
A.g $_{100.48}$ (+) x A.g $_{101.48}$ (-)	--	--	116	54.75
P.p (+) x P.p (-)	--	--	0.25	--
P.p (+) x A.g $_{6776a}$ (+)	--	--	1.5	--
P.p (+) x A.g $_{6776b}$ (-)	--	--	12.75	4.25
P.p (-) x A.g $_{6776a}$ (+)	--	--	8.5	2.5
P.p (-) x A.g $_{6776b}$ (-)	--	--	--	--
A.g $_{6776a}$ (+) x A.g $_{6776b}$ (-)	--	--	41.25	18.5

-- = No zygophores

Table 18: Effect of fungal extracts on the induction of zygophores in *Mucor mucedo.*
P.p = *P. parasitica*, B.t = *B. trispora*

Fungal extract (10 µg)	Average no. of zygophores of *Mucor mucedo* (+)		Average no. of zygophores of *Mucor mucedo* (-)	
	pH 2	pH 8	pH 2	pH 8
P. parasitica (+)	--	--	--	--
P. parasitica (-)	--	--	--	--
B. trispora (+)	51.5	65.5	157.5	176.25
B. trispora (-)	29.5	32.25	84.5	100.5
P.p (+) x P.p (-)	--	--	0.25	--
P.p (+) x B.t (+)	2.5	4.25	53.5	39.25
P.p (+) x B.t (-)	24.75	21.5	126.5	134.75
P.p (-) x B.t (+)	16.25	14.5	61.5	93.5
P.p (-) x B.t (-)	--	--	8.5	2.25
B.t (+) x B.t (-)	91.25	86.5	182.75	160.25

-- = No zygophores

Table 19: Extent of parasitic interaction development in crosses between *Parasitella parasitica* and different zygomycetes

Strains	*P. parasitica* (+)	*P. parasitica* (-)
Absidia glauca $_{100.48}$ (+)	--	L.i, Sk
Absidia glauca $_{101.48}$ (-)	S.i, Sk	--
Absidia glauca $_{6776a}$ (+)	--	L.i, Sk
Absidia glauca $_{6776b}$ (-)	S.i, Sk	--
Absidia orchidis	--	--
Absidia parricida	S.i, Sk	S.i, Sk
Absidia spinosa	--	--
Actinomucor elegans	L.i, Sk	L.i
Blakeslea trispora (+)	S.i, Sk	S.i, Sk
Blakeslea trispora (-)	S.i, Sk	S.i, Sk
Chaetocladium brefeldii	S.i, Sk	L.i
Coemansia formosensis	--	--
Cunninghamella elegans	--	--
Gilbertella persicaria	S.i, Sk	S.i, Sk
Gongronella butleri	L.i	--
Halteromyces radiatus	--	--
Linderina macrospora	L.i	S.i, Sk
Mortierella gamsii (+)	--	--
Mortierella gamsii (-)	--	--
Mortierella globulifera (+)	--	--
Mortierella globulifera (-)	--	--
Mortierella humilis (+)	--	--
Mortierella humilis (-)	--	--
Mortierella indohii (+)	--	--
Mortierella indohii (-)	--	--
Mortierella minutissima (+)	--	--
Mortierella minutissima (-)	--	--
Mortierella parvispora (+)	--	--
Mortierella parvispora (-)	--	--
Mucor mucedo (+)	L.i, Sk	--
Mucor mucedo (-)	S.i, Sk	S.i, Sk
Mucor racemosus (+)	S.i, Sk	S.i, Sk
Mucor racemosus (-)	S.i, Sk	S.i, Sk
Mycotypha africana	S.i, Sk	S.i, Sk
Phycomyces blakesleeanus (+)	--	--
Phycomyces blakesleeanus (-)	--	--
Pilaira anomala	--	--
Syncephalastrum racemosum	--	--
Syzigites megalocarpus	--	--
Thamnidium elegans (+)	S.i, Sk	S.i, Sk
Thamnidium elegans (-)	S.i, Sk	L.i, Sk
Thamnostylum piriforme	--	--
Zygorhynchus moelleri	S.i, Sk	S.i, Sk

L.i = Little interaction, S.i = Strong interaction, Sk = Sikyospore formation, -- = No interaction
Little interaction means, little numbers of mycelia were parasitically interacted.
Strong interaction means, big numbers of mycelia were parasitically interacted.

Table 20: Extent of sexual development in different species of zygomycetes

Strains	Extent of sexual interaction
Absidia glauca $_{100.48}$ (+) x *Absidia glauca* $_{101.48}$ (-)	Zygospore
Absidia glauca $_{6776a}$ (+) x *Absidia glauca* $_{6776b}$ (-)	Zygospore
Blakeslea trispora (+) x *Blakeslea trispora* (-)	Zygospore
Mortierella humilis (+) x *Mortierella humilis* (-)	Zygospore
Mortierella indohii (+) x *Mortierella indohii* (-)	Zygospore
Mortierella parvispora (+) x *Mortierella parvispora* (-)	Zygospore
Mucor mucedo (+) x *Mucor mucedo* (-)	Zygospore
Mucor racemosus (+) x *Mucor racemosus* (-)	Zygospore
Parasitella parasitica (+) x *Parasitella parasitica* (-)	Zygospore
Phycomyces blakesleeanus (+) x *Phycomyces blakesleeanus* (-)	Progametangia only
Thamnidium elegans (+) x *Thamnidium elegans* (-)	Zygospore
Absidia parricida (homothallic)	Zygospore
Zygorhynchus moelleri (homothallic)	Zygospore

Legend of thin layer chromatography plates

Lane	Heterothallic	Homothallic
1	*B. trispora* (+ -) pH 8	*B. trispora* (+ -) pH 8
2	P.p (+) x P.p (-) pH 8	P.p (+) x P.p (-) pH 8
3	P.p (+) x P.p (-) pH 2	P.p (+) x P.p (-) pH 2
4	Strain (+ -) pH 8	P.p (+) x Strain pH 8
5	Strain (+ -) pH 2	P.p (+) x Strain pH 2
6	P.p (+) x Strain (-) pH 8	P.p (-) x Strain pH 8
7	P.p (+) x Strain (-) pH 2	P.p (-) x Strain pH 2
8	P.p (-) x Strain (+) pH 8	Strain pH 8
9	P.p (-) x Strain (+) pH 2	Strain pH 2
10	Strain (+) pH 8	*P. parasitica* (+) pH 8
11	Strain (-) pH 8	*P. parasitica* (-) pH 8
12	Strain (+) pH 2	*P. parasitica* (+) pH 2
13	Strain (-) pH 2	*P. parasitica* (-) pH 2
14	*P. parasitica* (+) pH 8	*B. trispora* (+ -) pH 2
15	*P. parasitica* (-) pH 8	
16	*P. parasitica* (+) pH 2	
17	*P. parasitica* (-) pH 2	
18	*B. trispora* (+ -) pH 2	

P.p = *P. parasitica*

The thin layer chromatography of the heterothallic strains represented in Figures:

4, 5, 10, 18, 19, 20, 21, 22, 23, 24, 25, 27, 31.

The thin layer chromatography of the homothallic strains represented in Figures:

6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 26, 28, 29, 30, 32, 33.

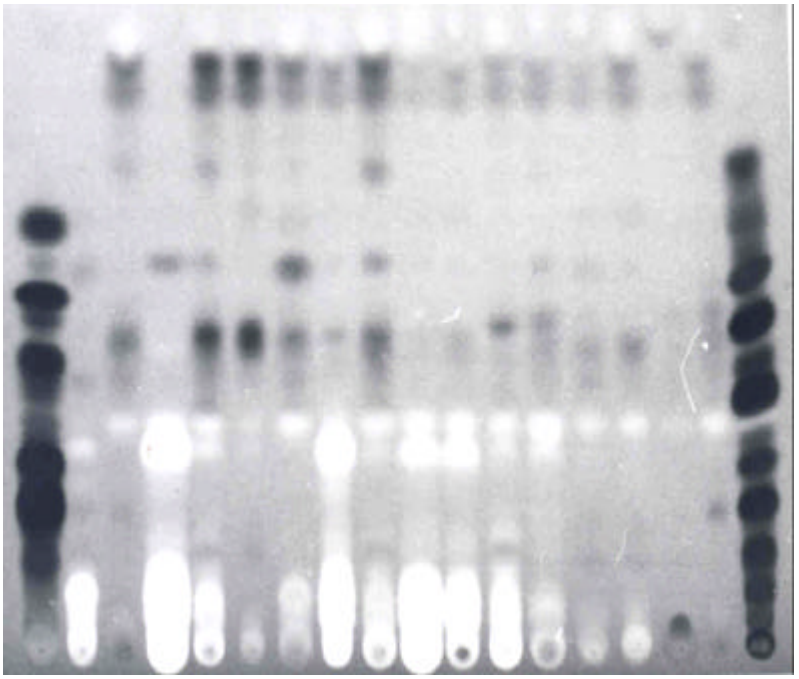


Fig. 4: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Absidia glauca* 100.48 (+), 101.48 (-) (heterothallic).

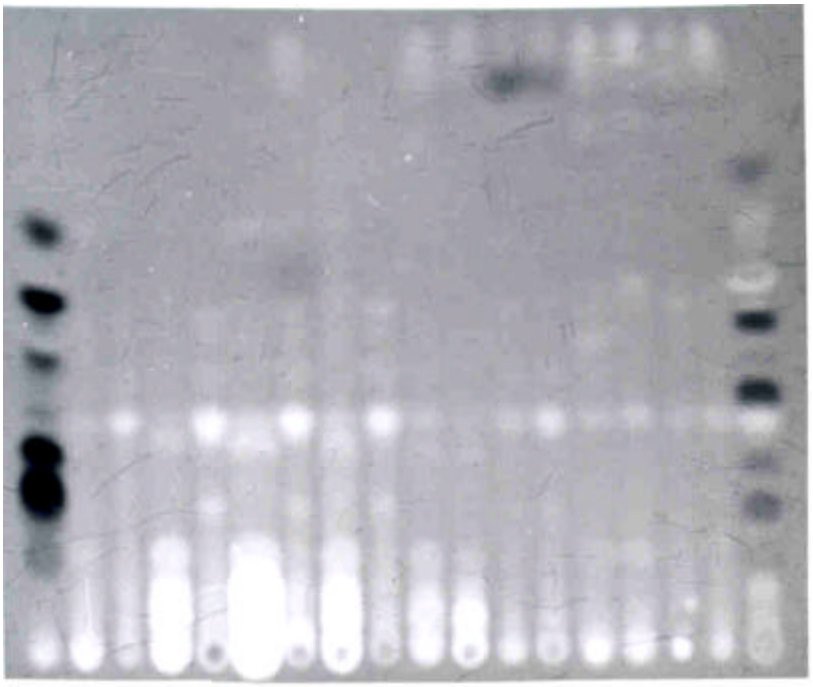


Fig. 5: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Absidia glauca* 6776 a (+), 6776 b (-) (heterothallic).

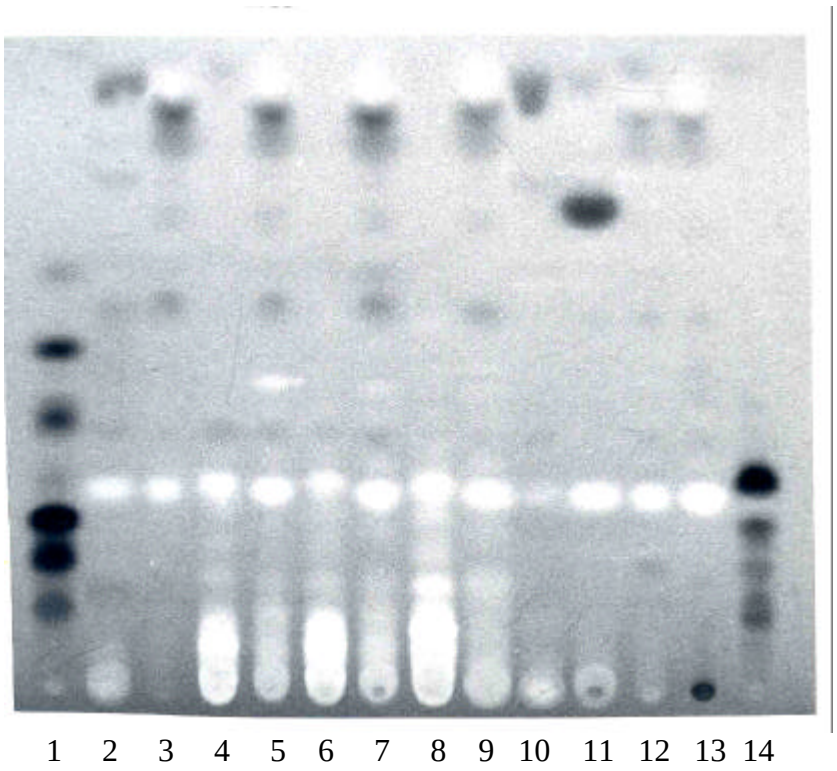


Fig. 6: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Absidia orchidis* (homothallic).

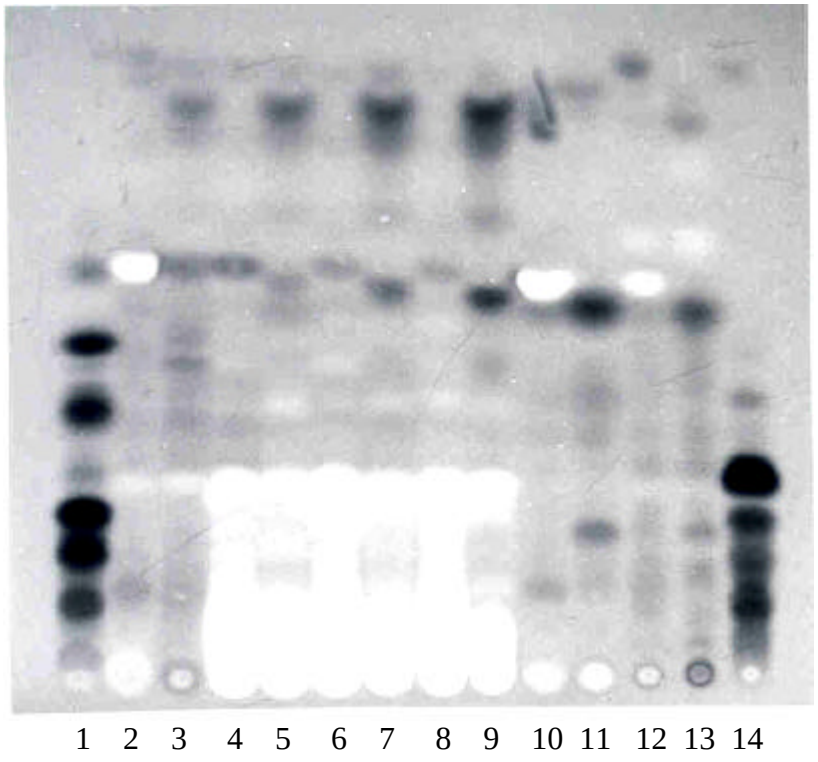
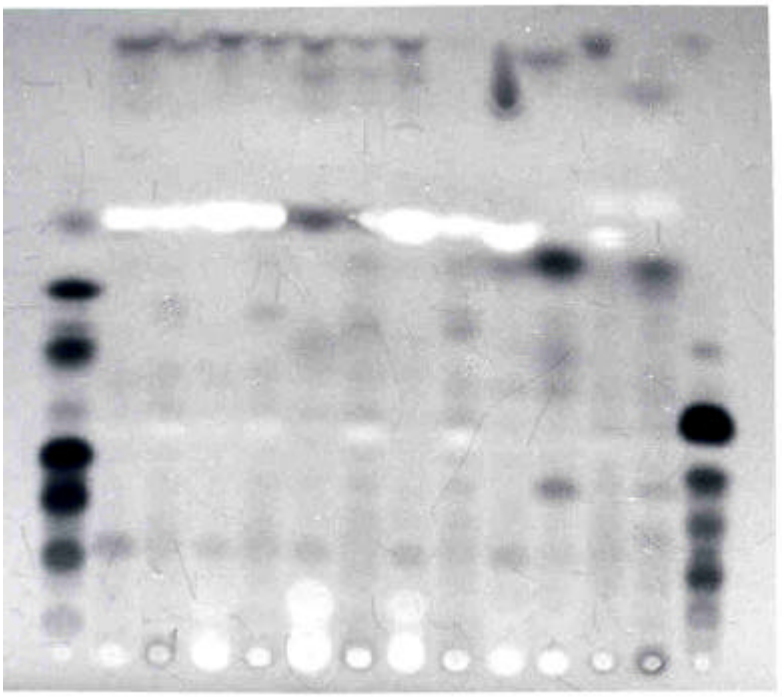
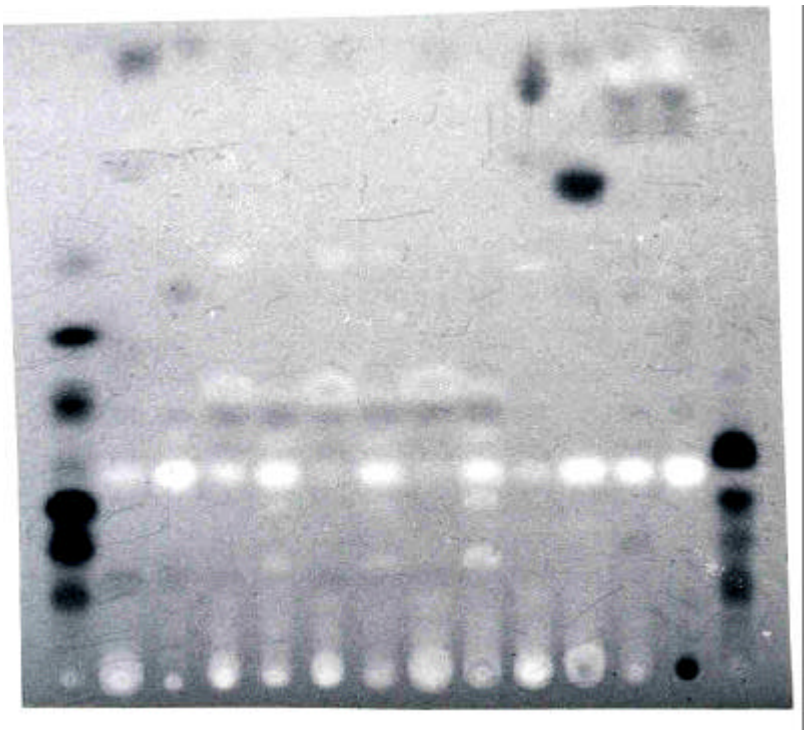


Fig. 7: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Absidia parricida* (homothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 8: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Absidia spinosa* (homothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 9: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Actinomucor elegans* (homothallic).

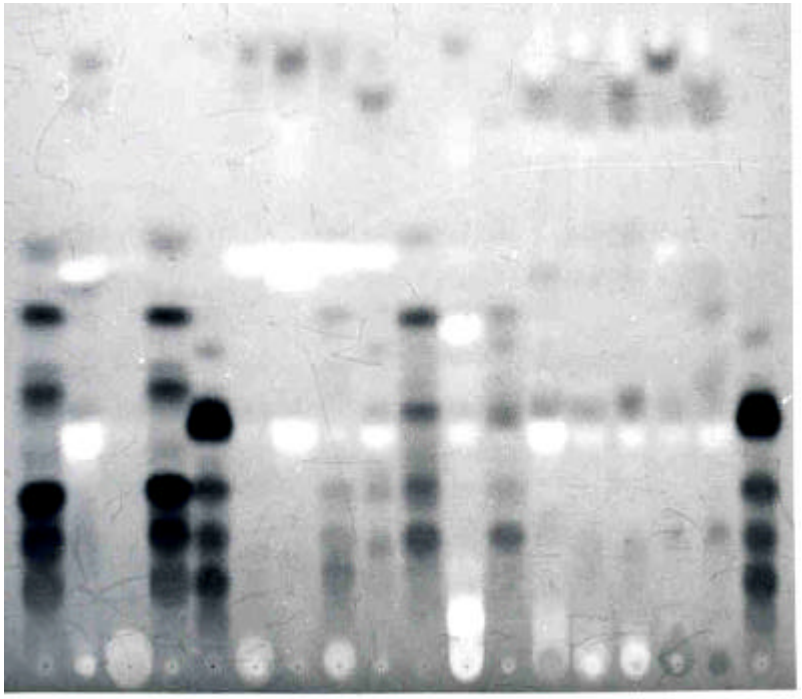


Fig. 10: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Blakeslea trispora* (heterothallic).

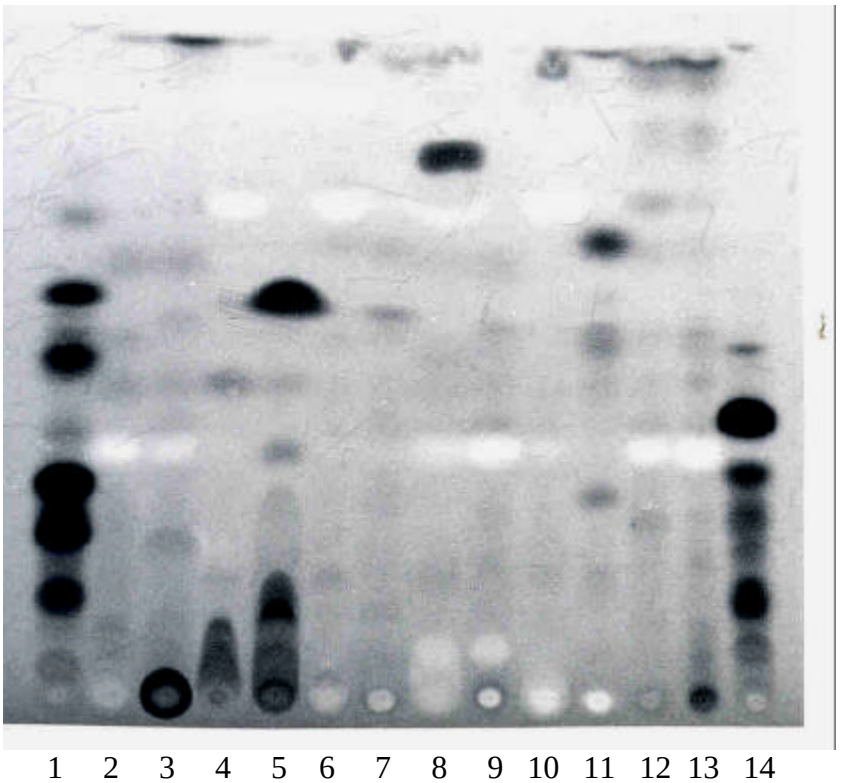
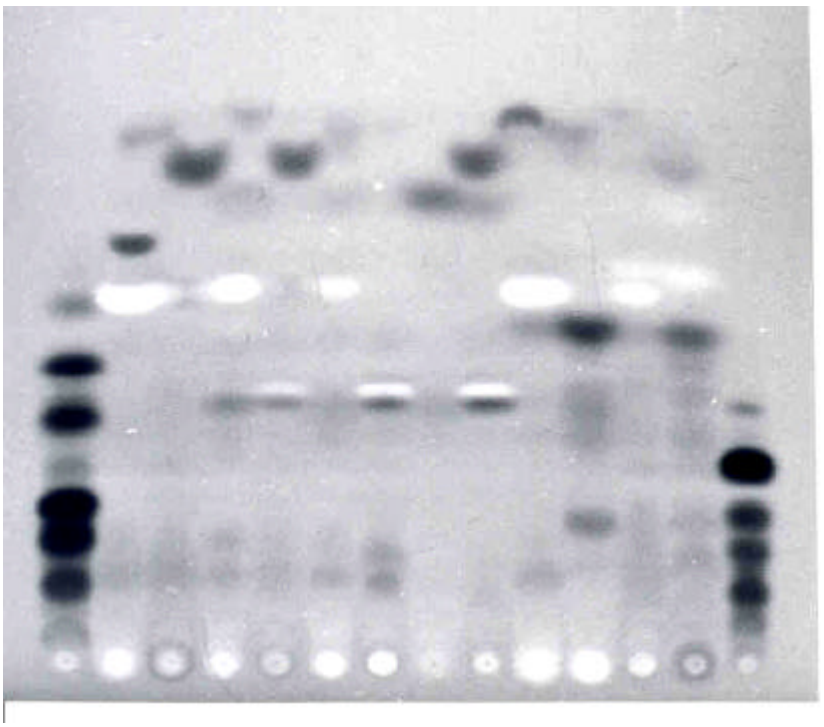
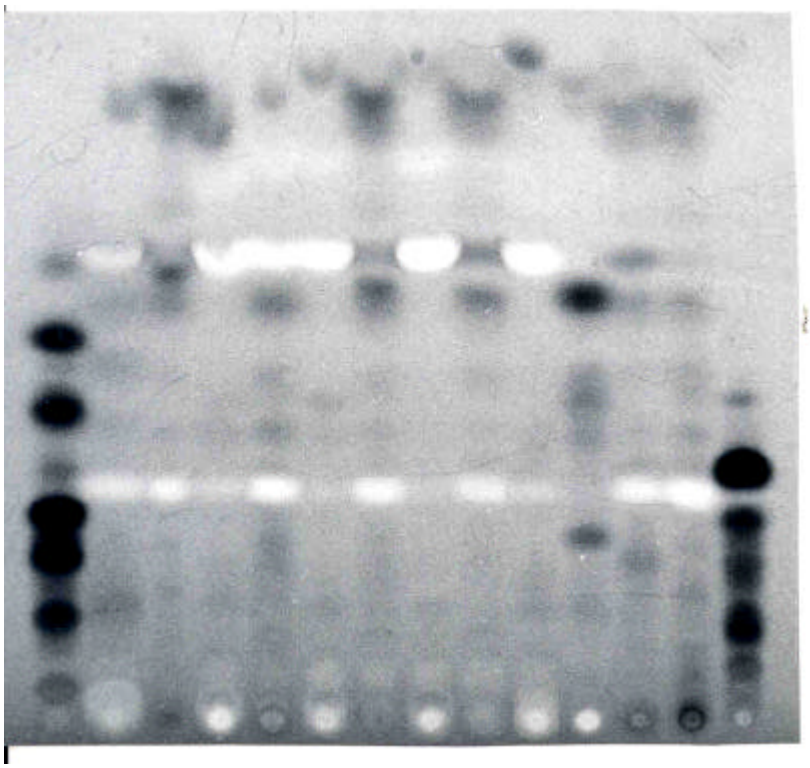


Fig. 11: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Chaetocladium brefeldii* (homothallic).



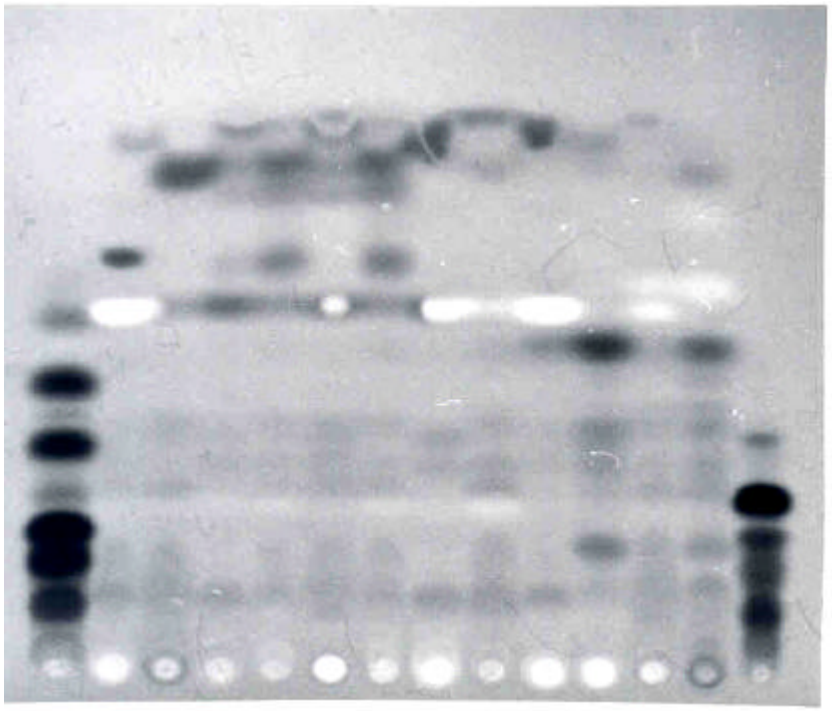
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 12: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Coemansia formosensis* (homothallic).



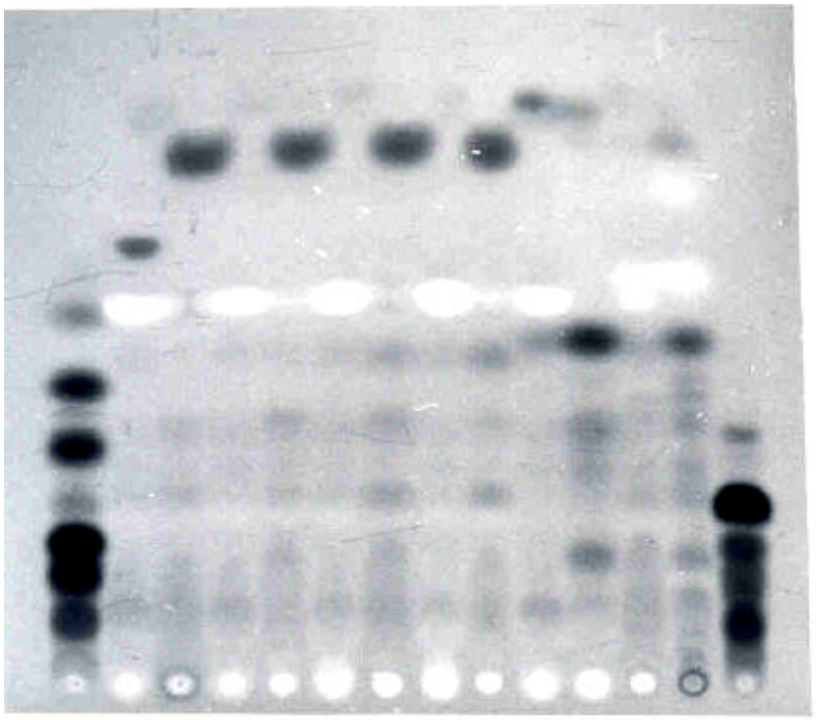
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 13: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Cunninghamella elegans* (homothallic).



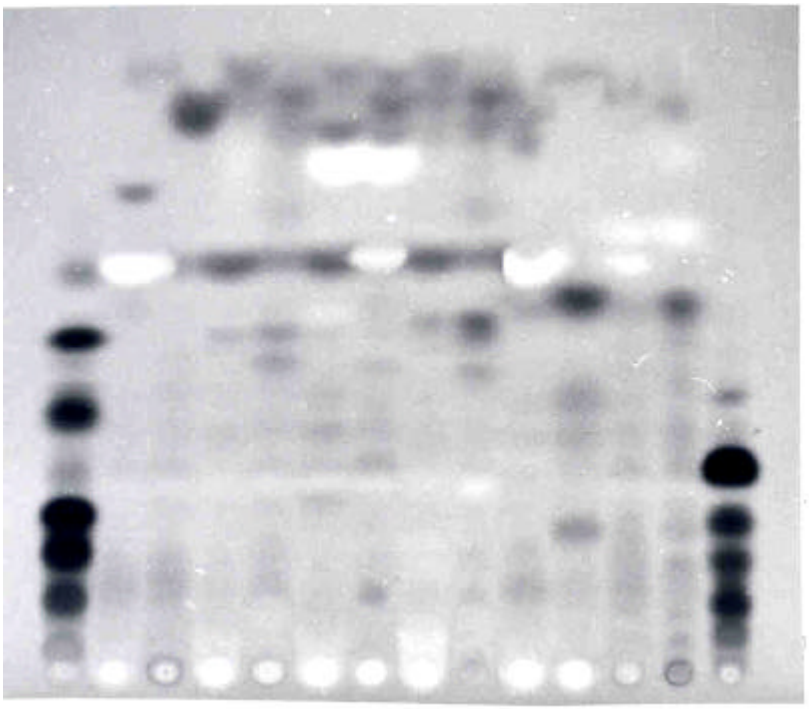
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 14: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Gilbertella persicaria* (homothallic).



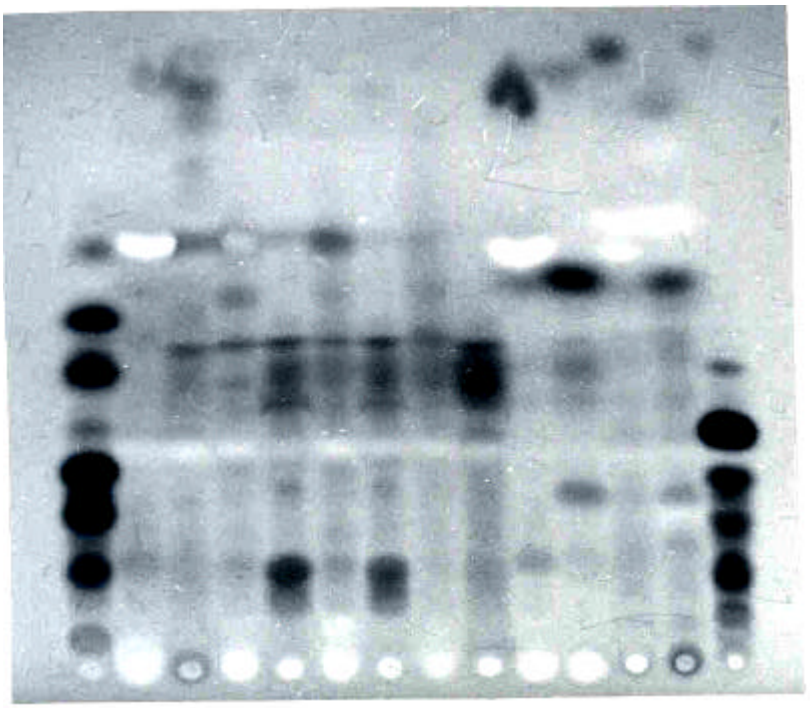
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 15: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Gongronella butleri* (homothallic).



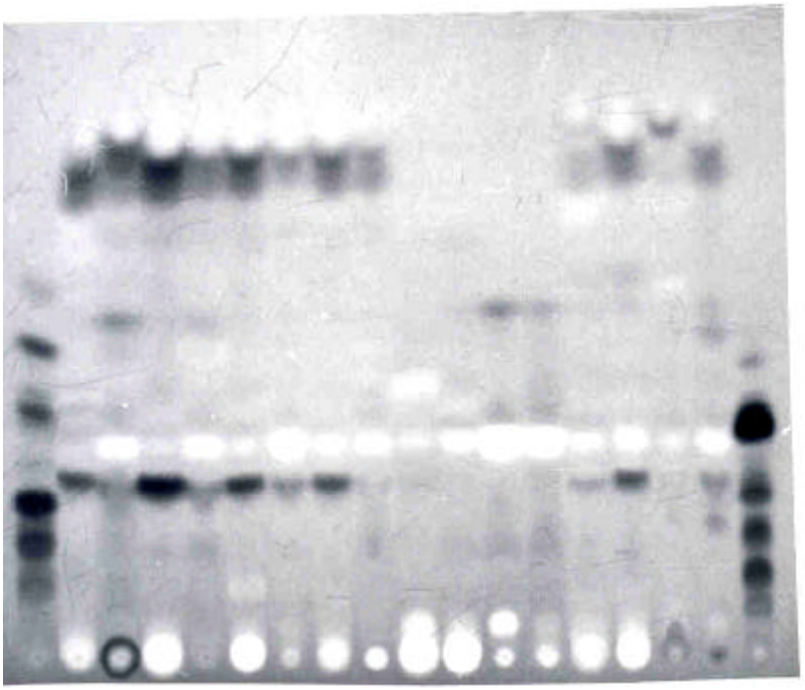
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 16: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Halteromyces radiatus* (homothallic).



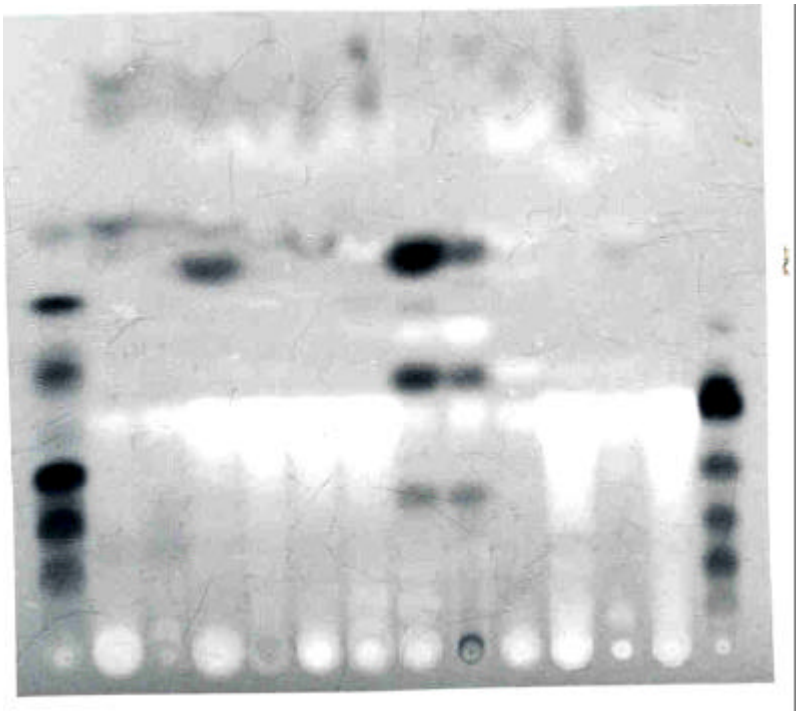
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 17: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Linderina macrospora* (homothallic).



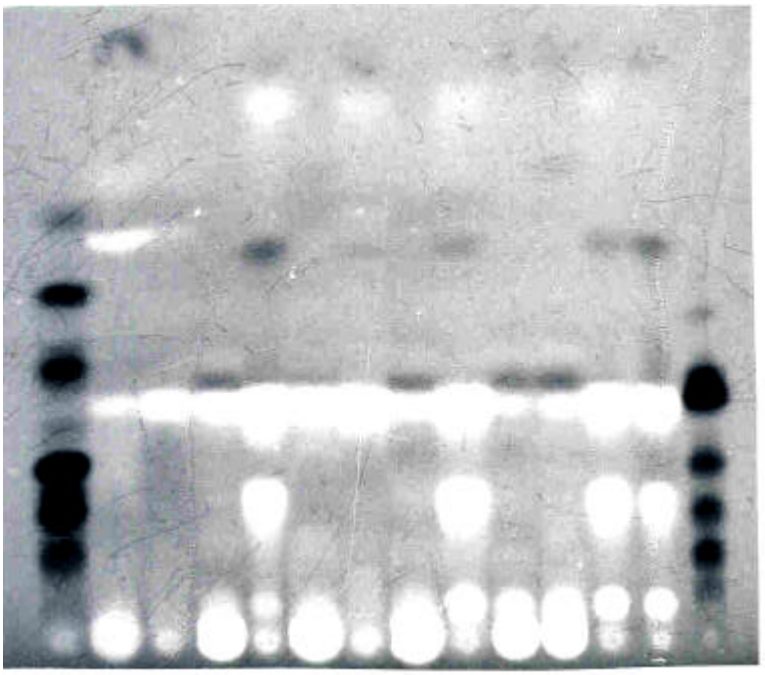
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 18: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella gamsii* (heterothallic).



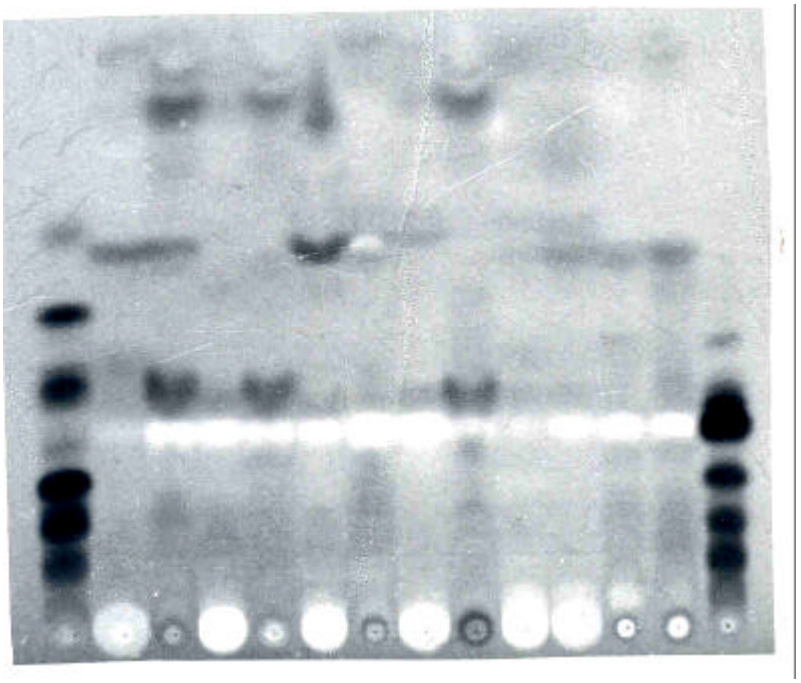
1 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 19: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella globulifera* (heterothallic).



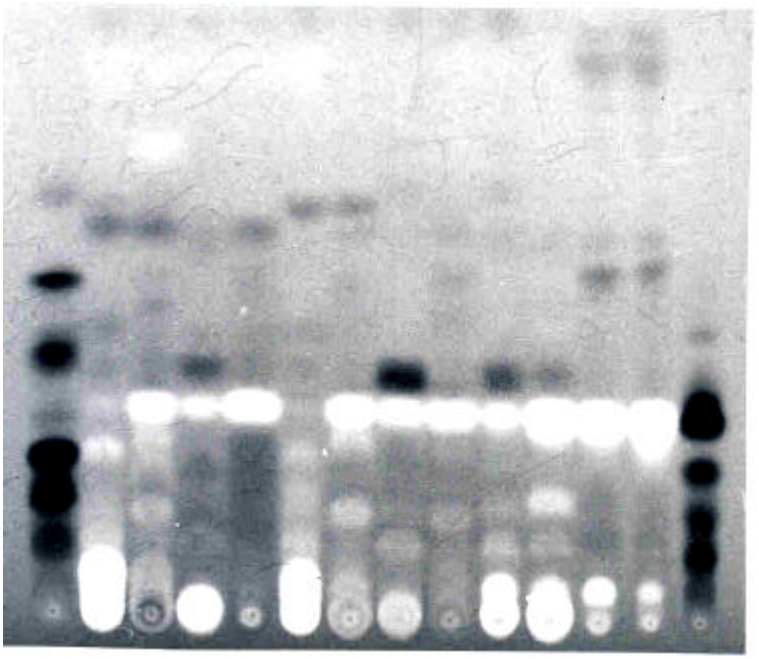
1 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 20: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella humilis* (heterothallic).



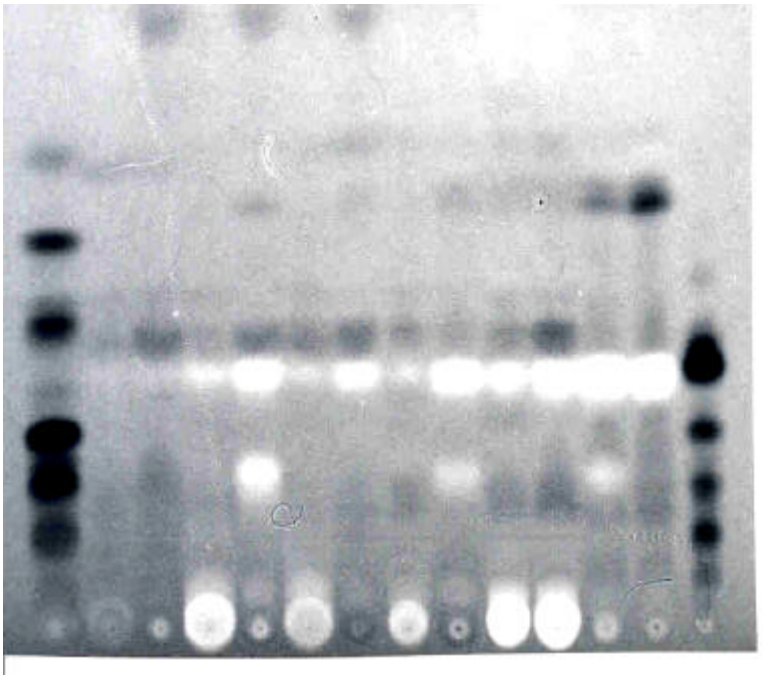
1 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 21: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella indohii* (heterothallic).



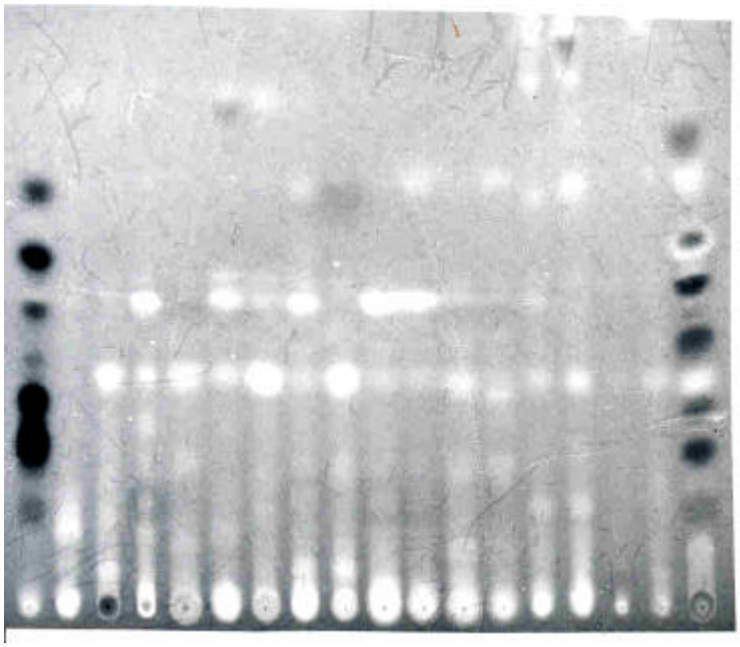
1 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 22: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella minutissima* (heterothallic).



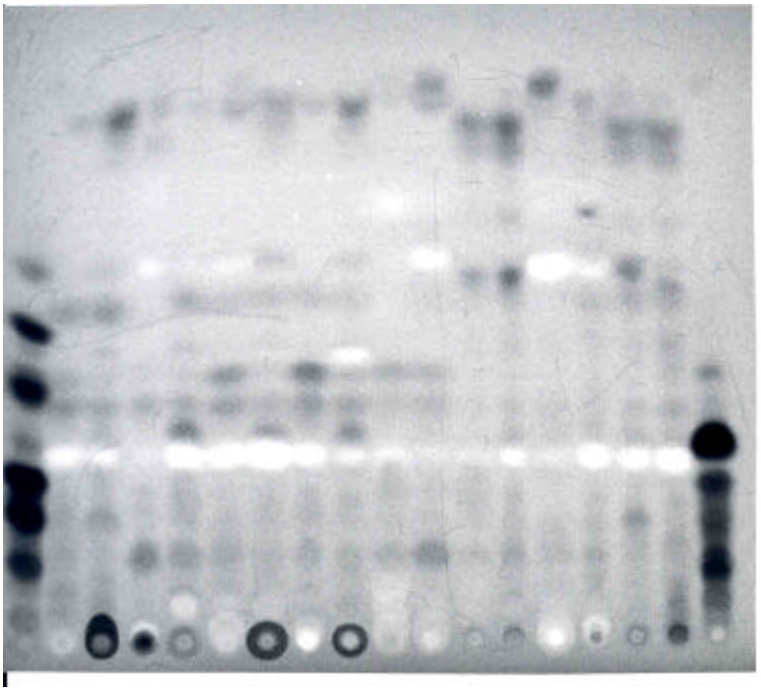
1 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 23: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mortierella parvispora* (heterothallic).



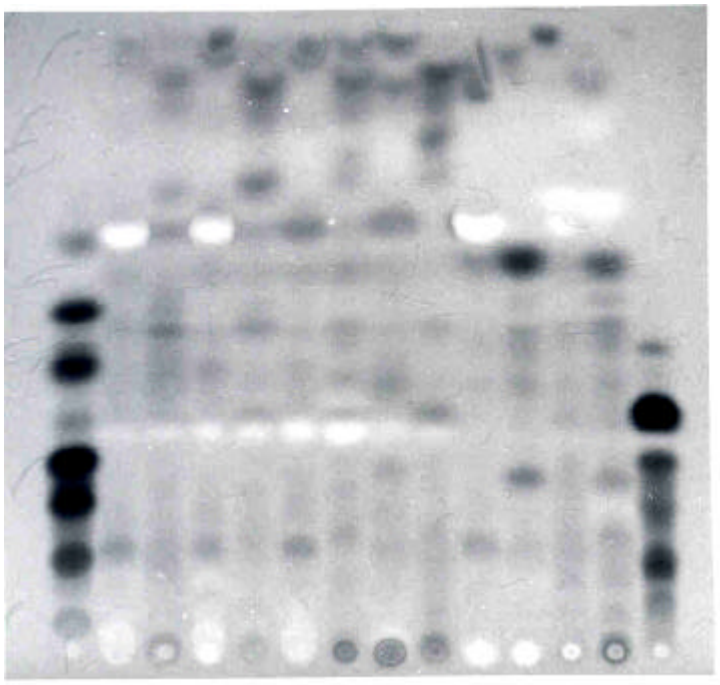
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 24: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mucor mucedo* (heterothallic).



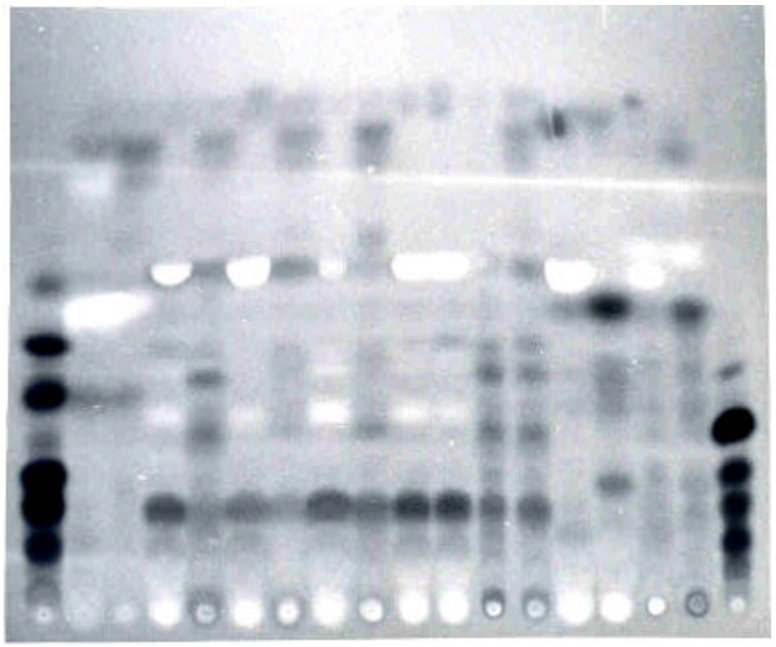
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 25: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mucor racemosus* (heterothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 26: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Mycotypha africana* (homothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 27: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Phycomyces blakesleeanus* (heterothallic).

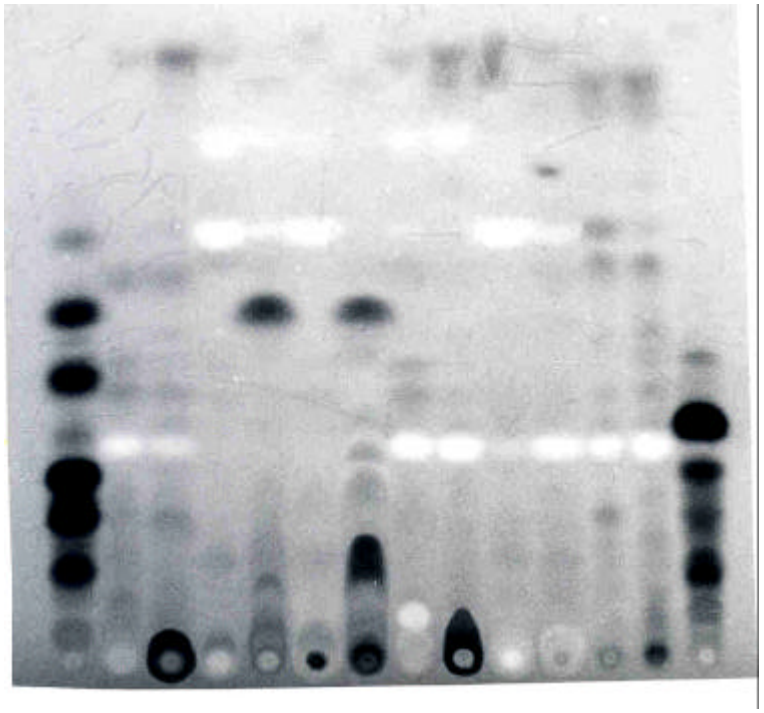


Fig. 28: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Pilaira anomala* (homothallic).

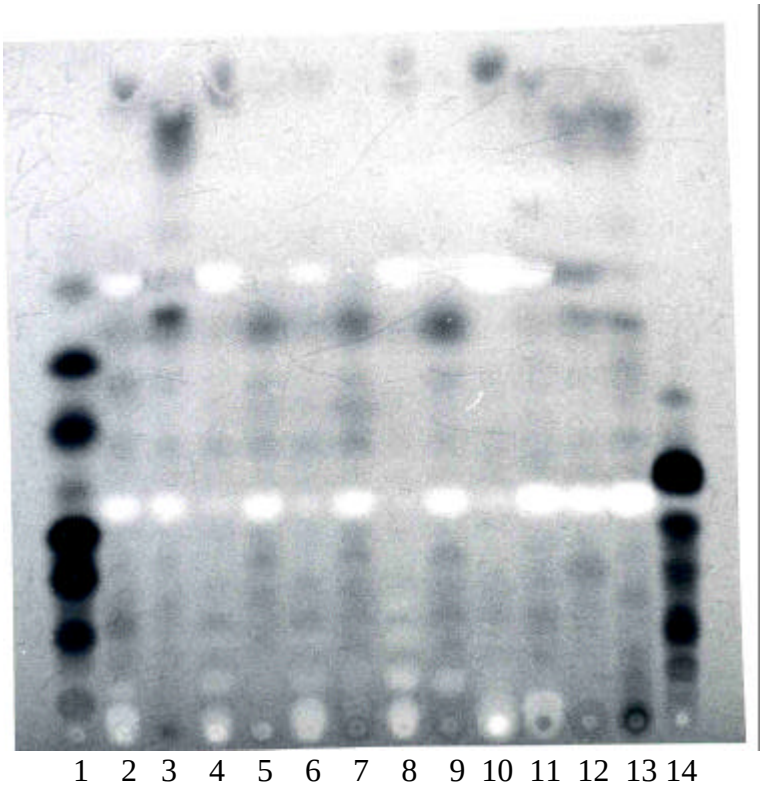
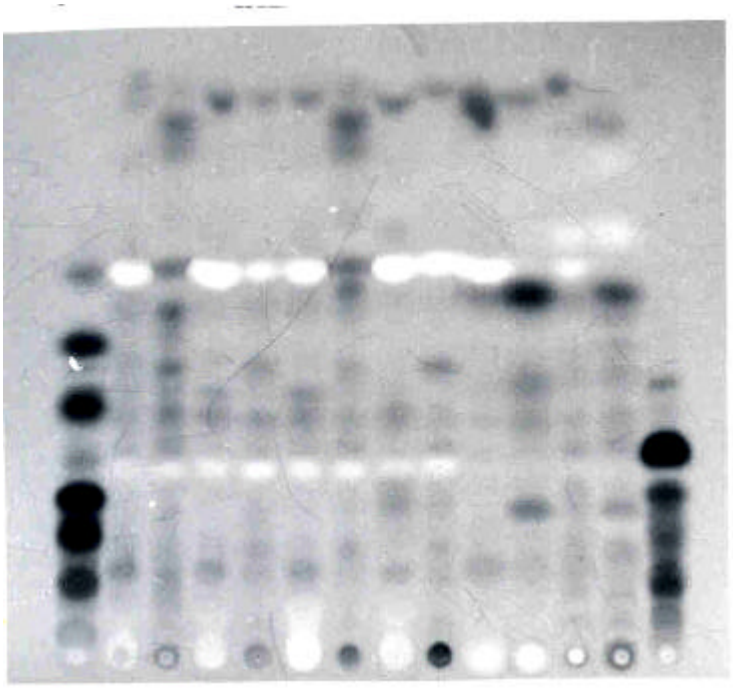
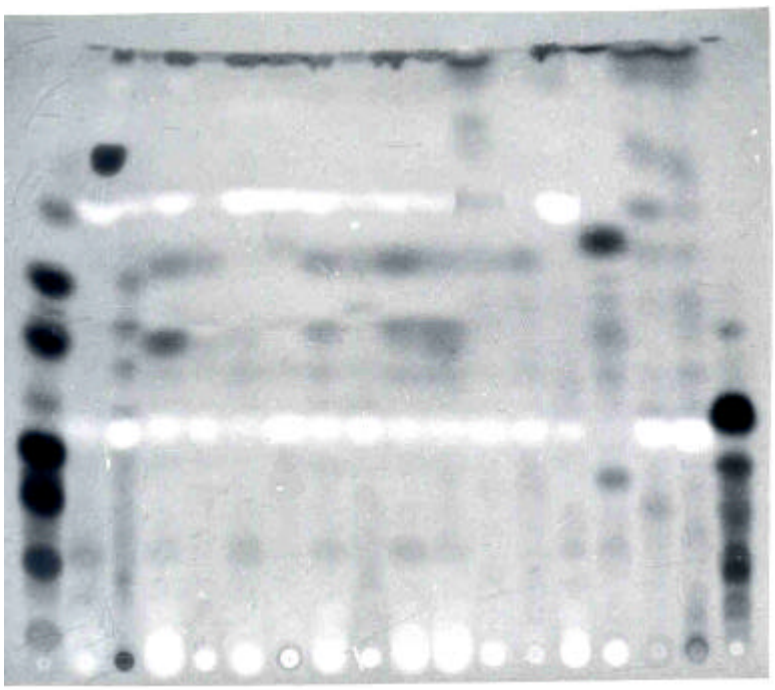


Fig. 29: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Syncephalastrum racemosum* (homothallic).



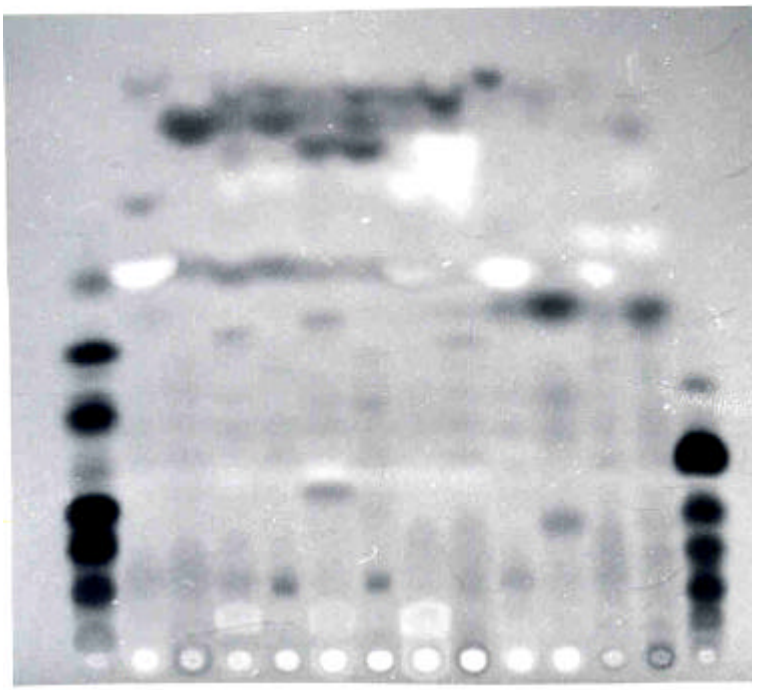
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 30: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Syzigites megalocarpus* (homothallic).



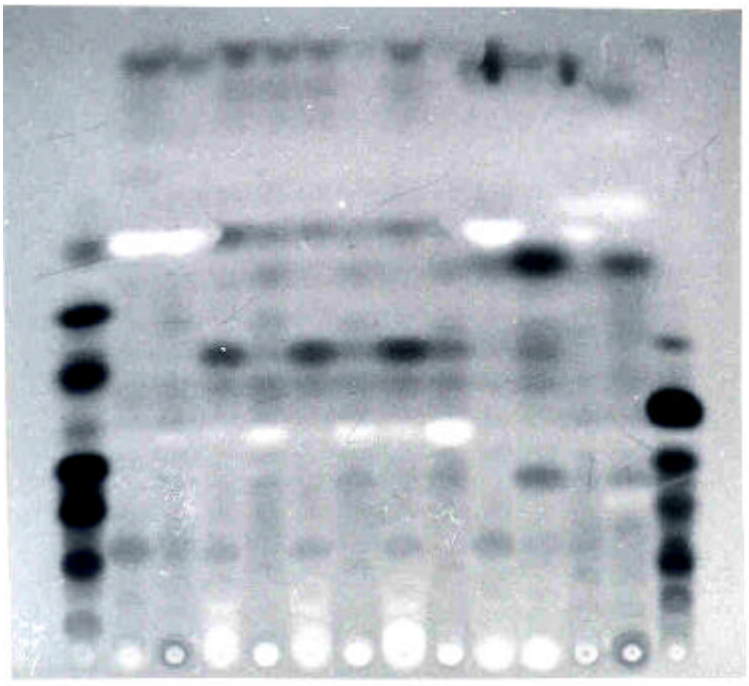
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Fig. 31: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Thamnidium elegans* (heterothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 32: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Thamnostylum piriforme* (homothallic).



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 33: Thin layer chromatograghic analysis of both single and combined culture extracts of *Parasitella parasitica* (heterothallic) and *Zygorhynchus moelleri* (homothallic).

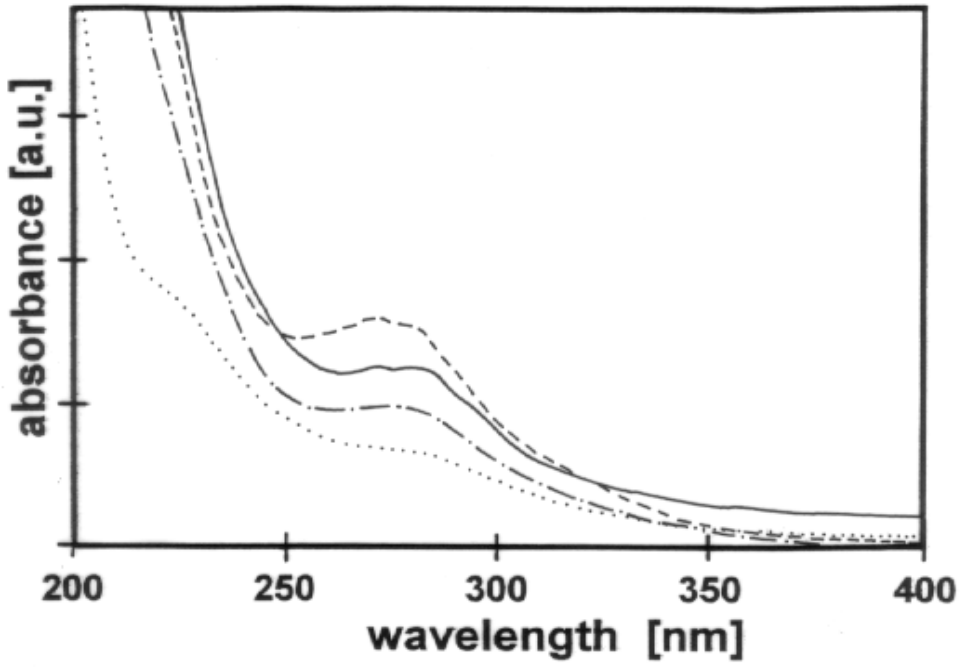


Fig. 34: UV-absorbance spectra of single culture extracts of *Parasitella parasitica*. (—) *P. parasitica* (+) pH 2, (---) *P. parasitica* (+) pH 8, (….) *P. parasitica* (-) pH 2, (-.-.-) *P. parasitica* (-) pH 8.

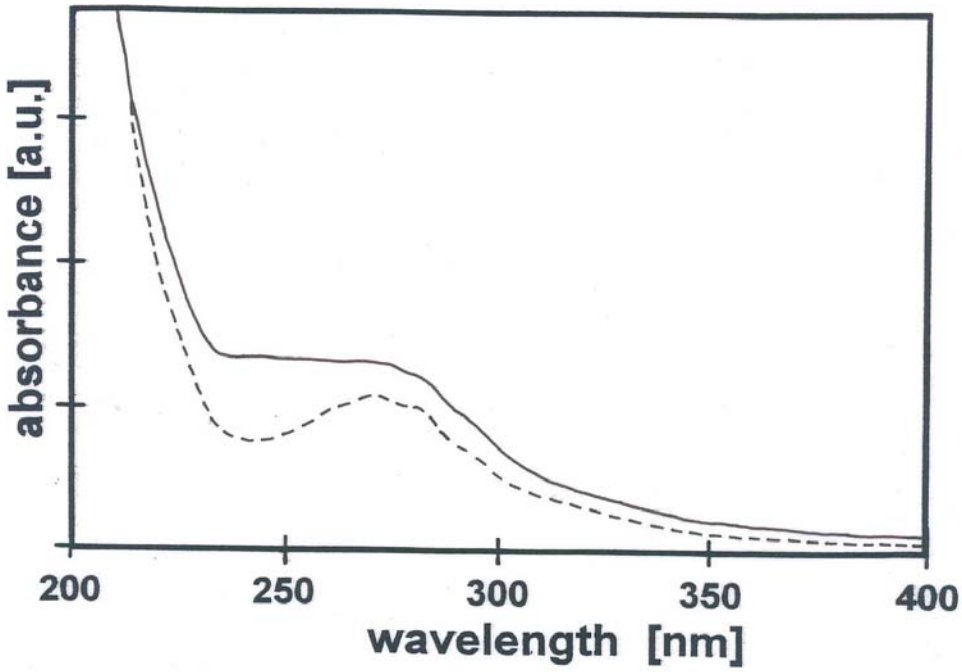


Fig. 35: UV-absorbance spectra of combined culture extracts of (+) and (-) mating types of *Parasitella parasitica*. (—) *P. parasitica* (+) x *P. parasitica* (-) pH 2, (---) *P. parasitica* (+) x *P. parasitica* (-) pH 8.

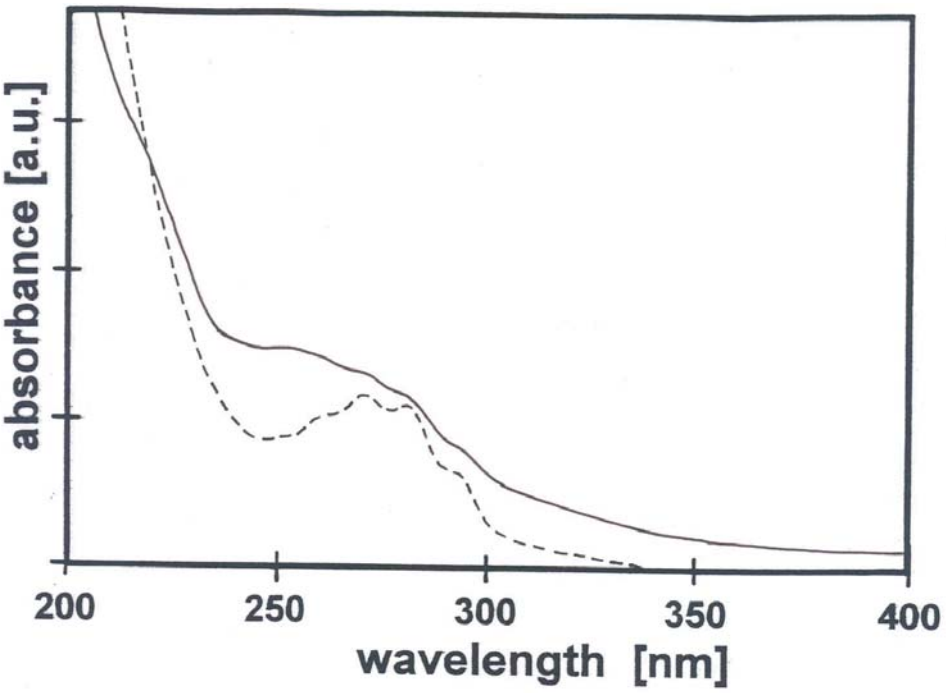


Fig. 36: UV-absorbance spectra of single culture extracts of *Absidia spinosa*.
(—) *A. spinosa* pH 2, (---) *A. spinosa* pH 8.

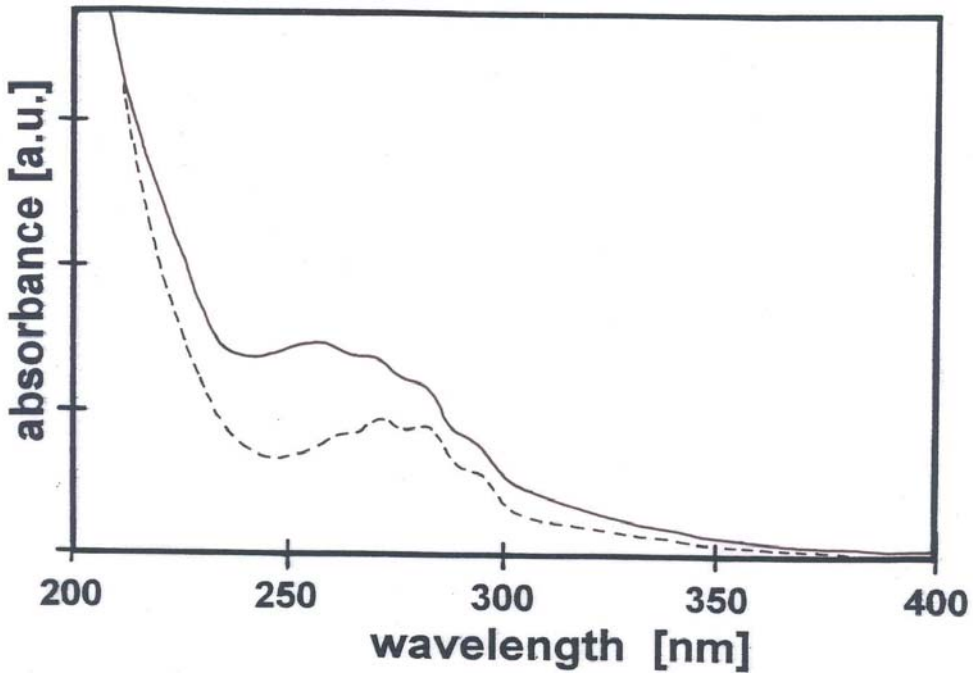


Fig. 37: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Absidia spinosa*. (—) *P. parasitica* (+) x *A. spinosa* pH 2,
(---) *P. parasitica* (+) x *A. spinosa* pH 8.

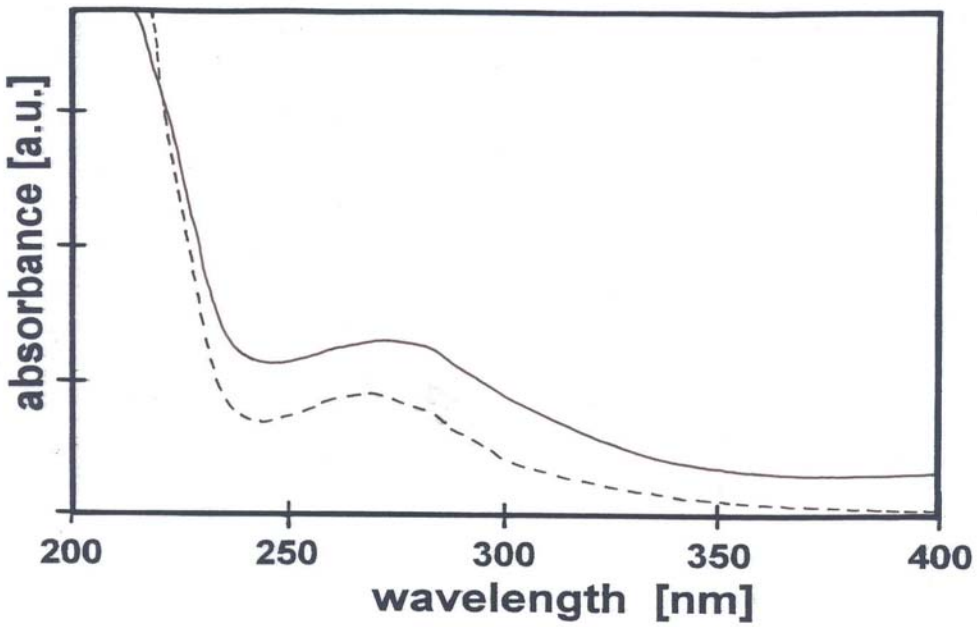


Fig. 38: UV-absorbance spectra of single culture extracts of *Actinomucor elegans*. (—) *A. elegans* pH 2, (---) *A. elegans* pH 8.

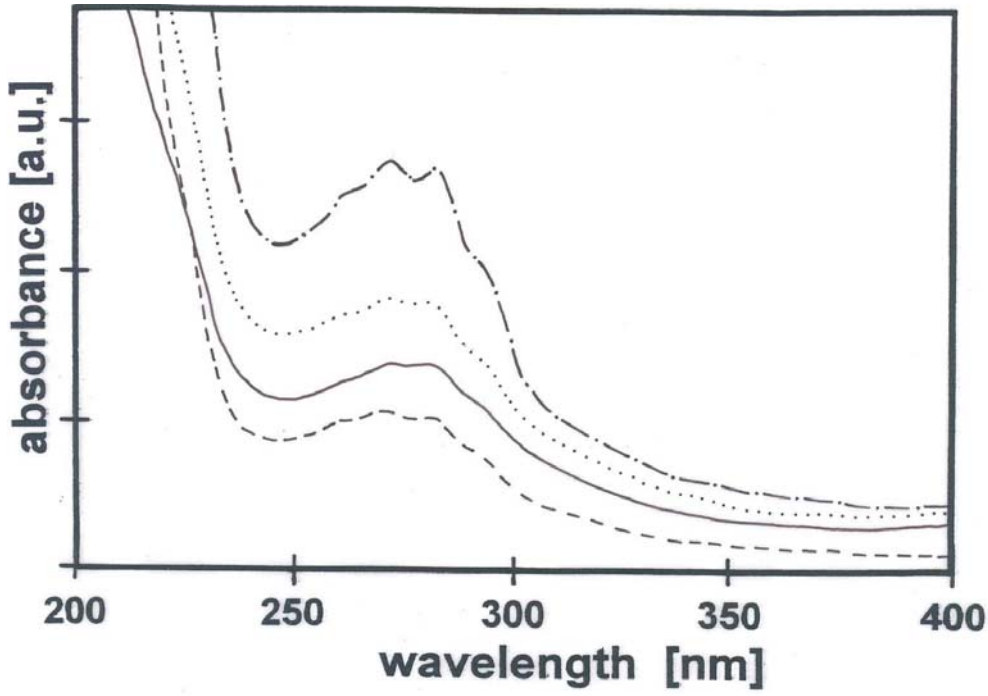


Fig. 39: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Actinomucor elegans*. (—) *P. parasitica* (+) x *A. elegans* pH 2, (---) *P. parasitica* (+) x *A. elegans* pH 8, (….) *P. parasitica* (-) x *A. elegans* pH 2, (-.-.-) *P. parasitica* (-) x *A. elegans* pH 8.

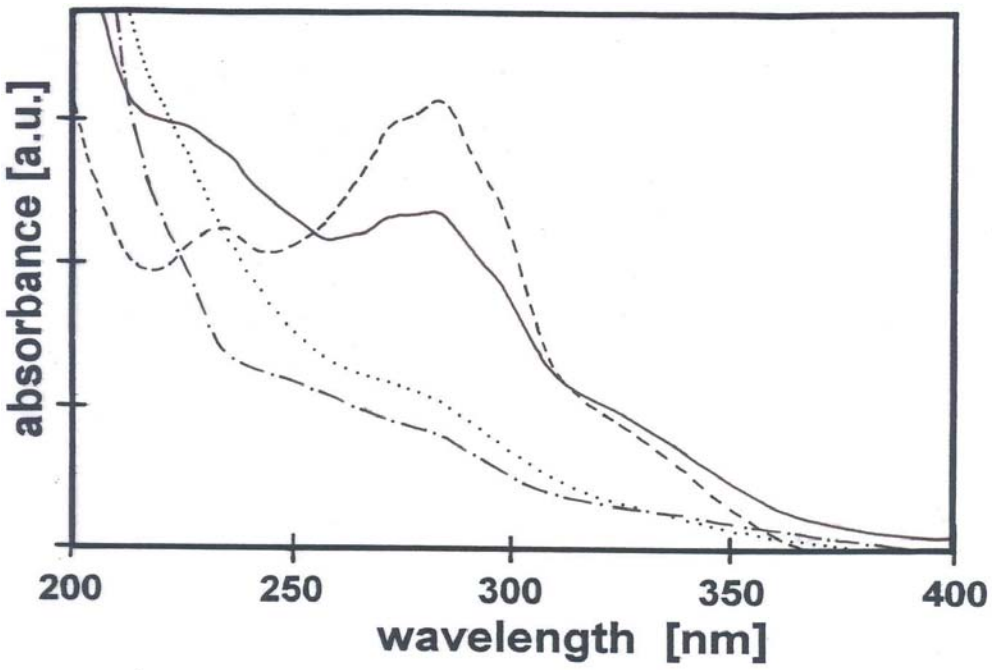


Fig. 40: UV-absorbance spectra of single culture extracts of *Blakeslea trispora*. (—) *B. trispora* (+) pH 2, (---) *B. trispora* (+) pH 8, (….) *B. trispora* (-) pH 2, (-.-.-) *B. trispora* (-) pH 8.

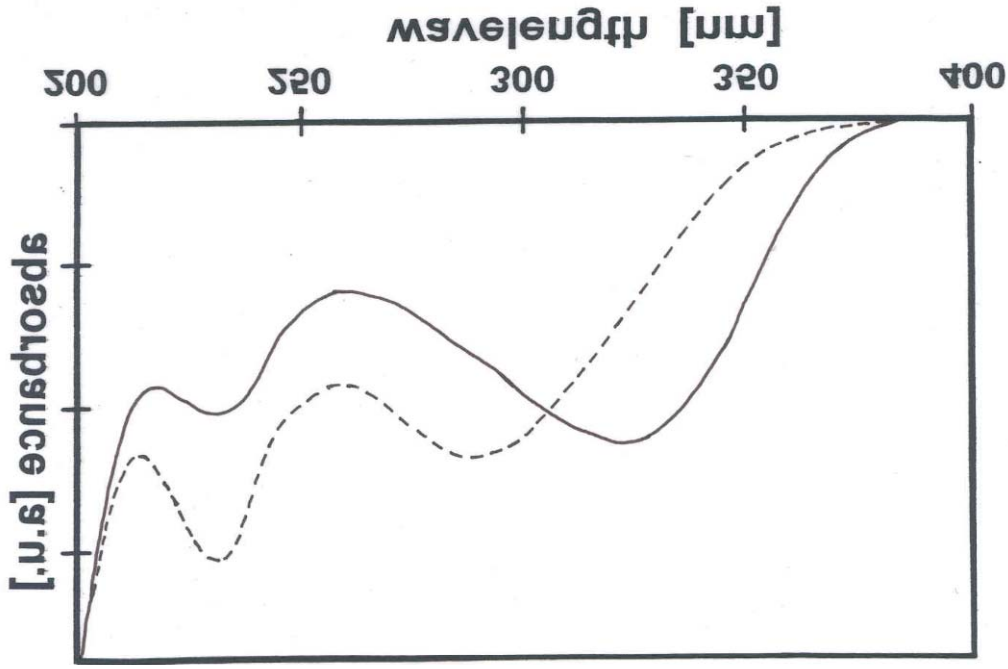


Fig. 41: UV-absorbance spectra of combined culture extracts of (+) and (-) mating types of *Blakeslea trispora.* (—) *B. trispora* (+) x *B. trispora* (-) pH 2, (---) *B. trispora* (+) x *B. trispora* (-) pH 8.

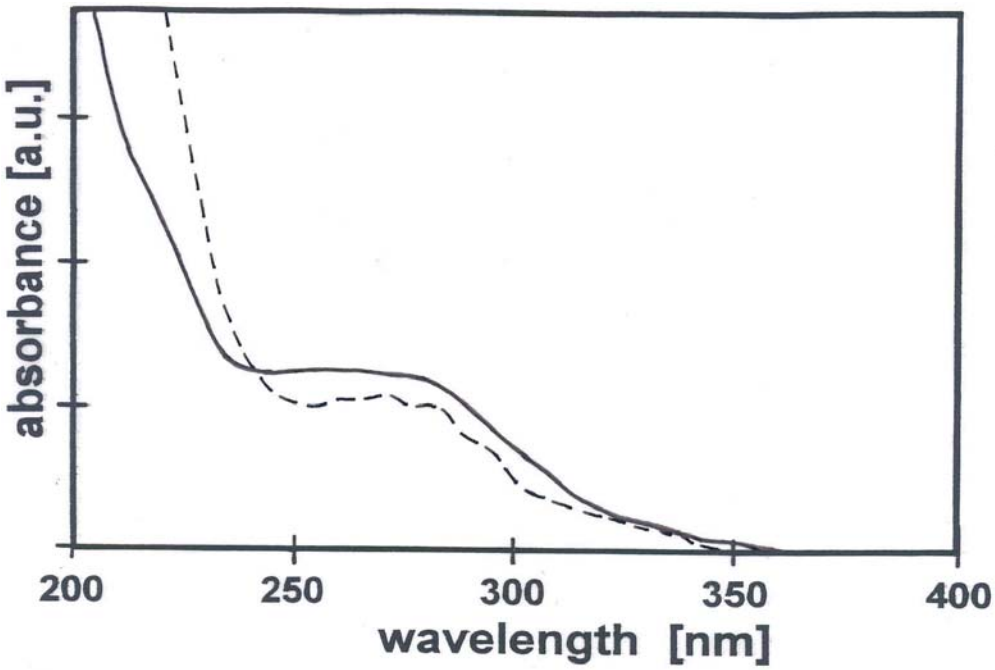


Fig. 42: UV-absorbance spectra of single culture extracts of *Chaetocladium brefeldii*. (—) *C. brefeldii* pH 2, (---) *C. brefeldii* pH 8.

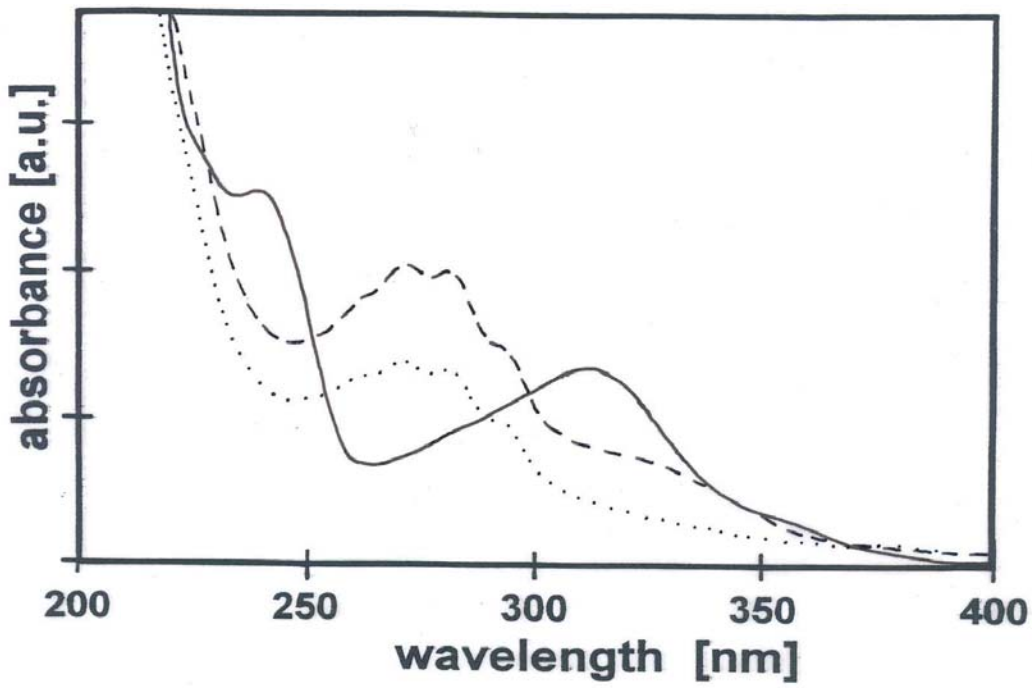


Fig. 43: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and C*haetocldium brefeldii*. (—) *P. parasitica* (+) x *C. brefeldii* pH 2, (---) *P. parasitica* (+) x *C. brefeldii* pH 8, (....) *P. parasitica* (-) x *C. brefeldii* pH 8.

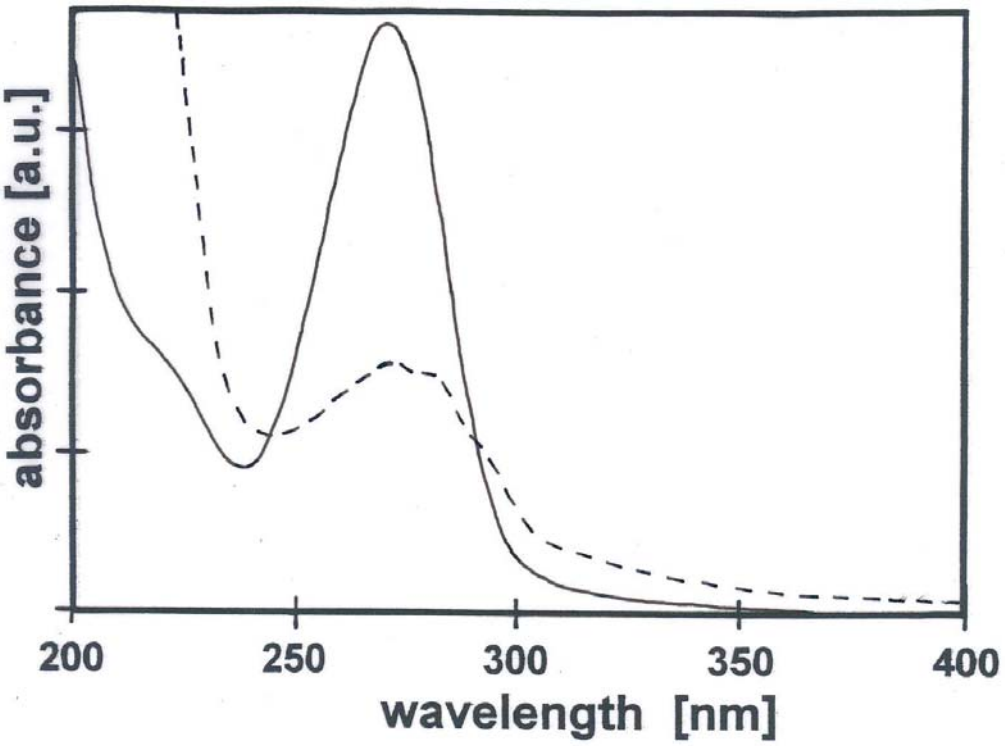


Fig. 44: UV-absorbance spectra of pH 8 single culture extract of *Coemansia formosensis* (—) and pH 8 combined culture extract of *P. parasitica* (+) x *C. formosensis* (---).

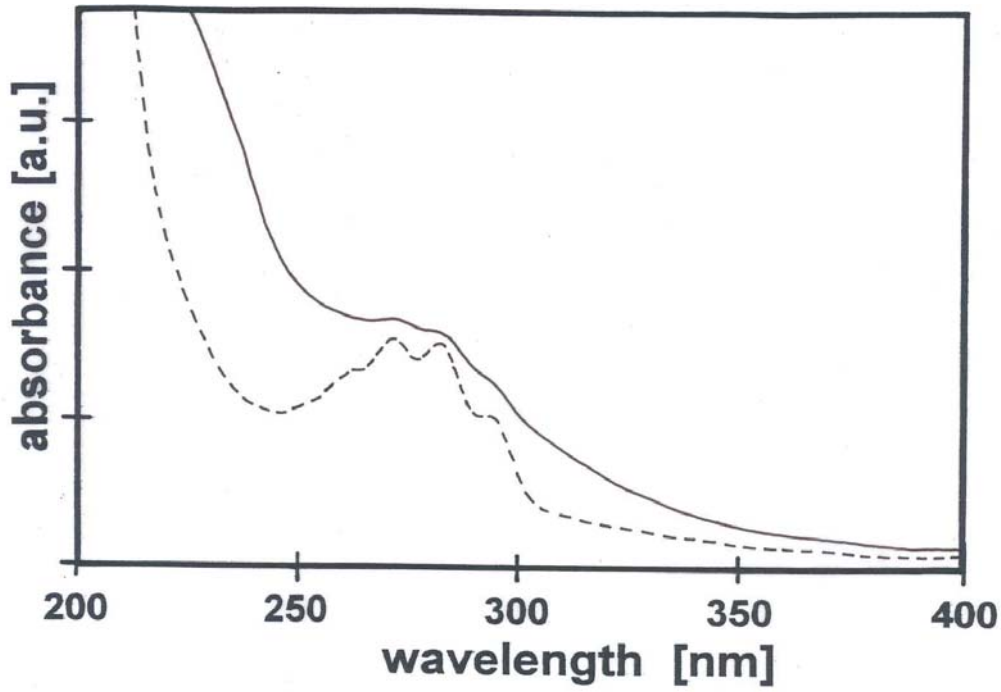


Fig. 45: UV-absorbance spectra of single culture extracts of *Cunninghamella elegans*. (—) *C. elegans* pH 2, (---) *C. elegans* pH 8.

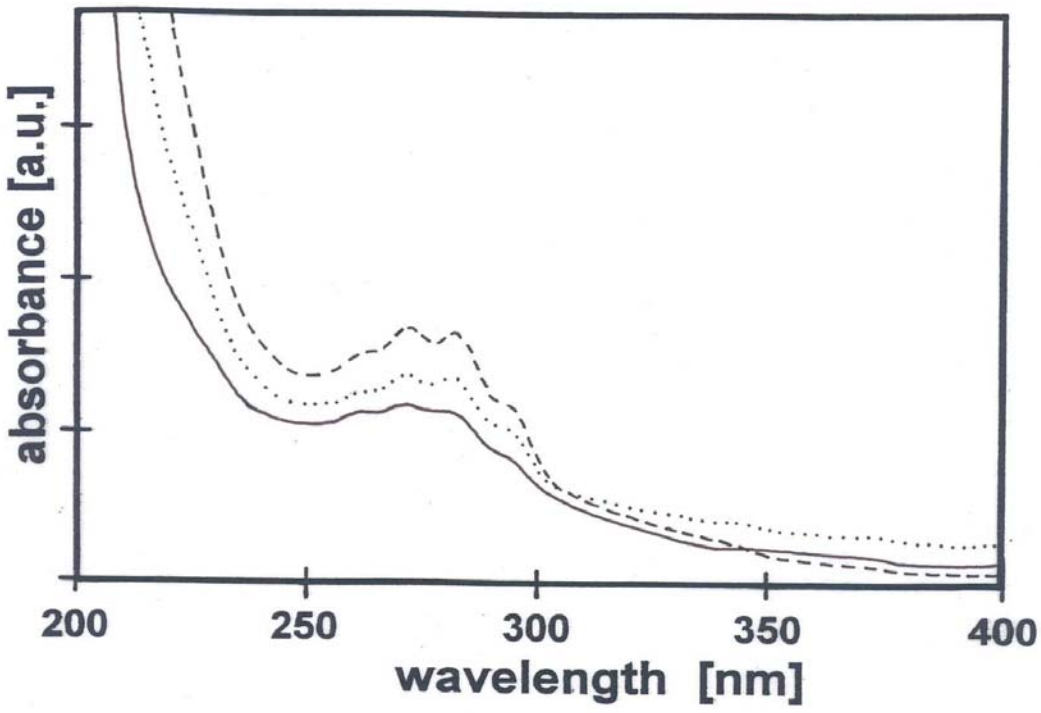


Fig. 46: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and C*unninghamella elegans*. (—) *P. parasitica* (+) x *C. elegans* pH 2, (---) *P. parasitica* (+) x *C. elegans* pH 8, (....) *P. parasitica* (-) x *C. elegans* pH 8.

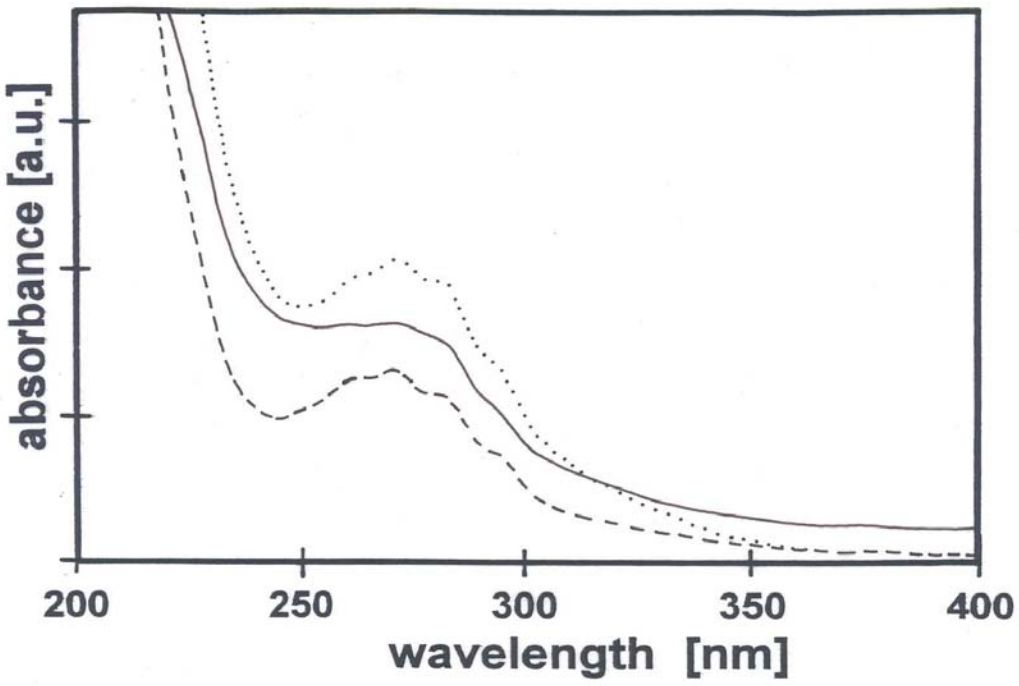


Fig. 47: UV-absorbance spectra of pH 2 (—) and pH 8 (---) single culture extracts of *Gilbertella persicaria* and pH 8 combined culture extract of. *P. parasitica* (+) x *G. persicaria* (....).

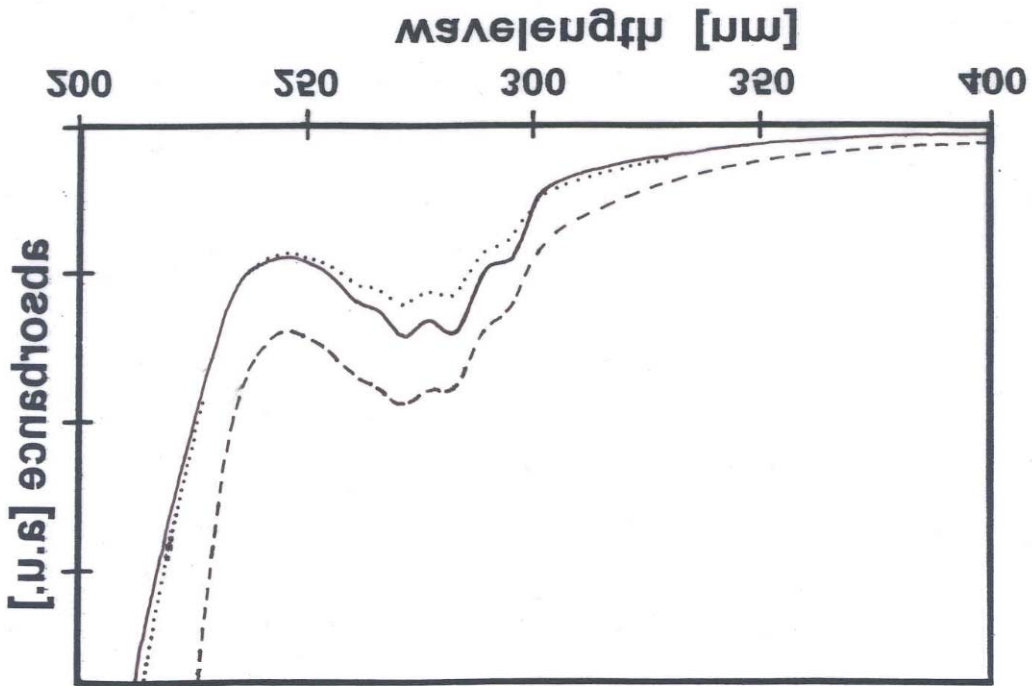


Fig. 48: UV-absorbance spectra of pH 8 single culture extract of *Gongronella butleri* (—), pH 8 combined culture extract of *P. parasitica* (+) x *G. butleri* (---) and pH 8 combined culture extract of *P. parasitica* (-) x *G. butleri* (....).

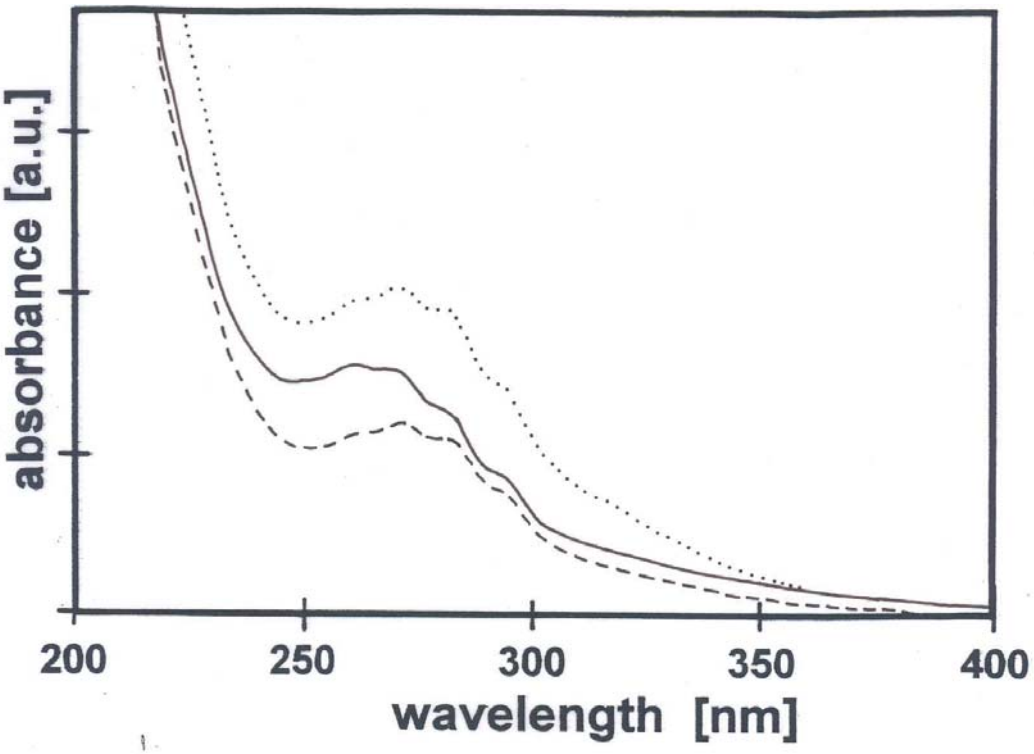


Fig. 49: UV-absorbance spectra of pH 8 single culture extract of *Halteromyces radiatus* (—), pH 8 combined culture extract of *P. parasitica* (+) x *H. radiatus* (---) and pH 8 combined culture extract of *P. parasitica* (-) x *H. radiatus* (....).

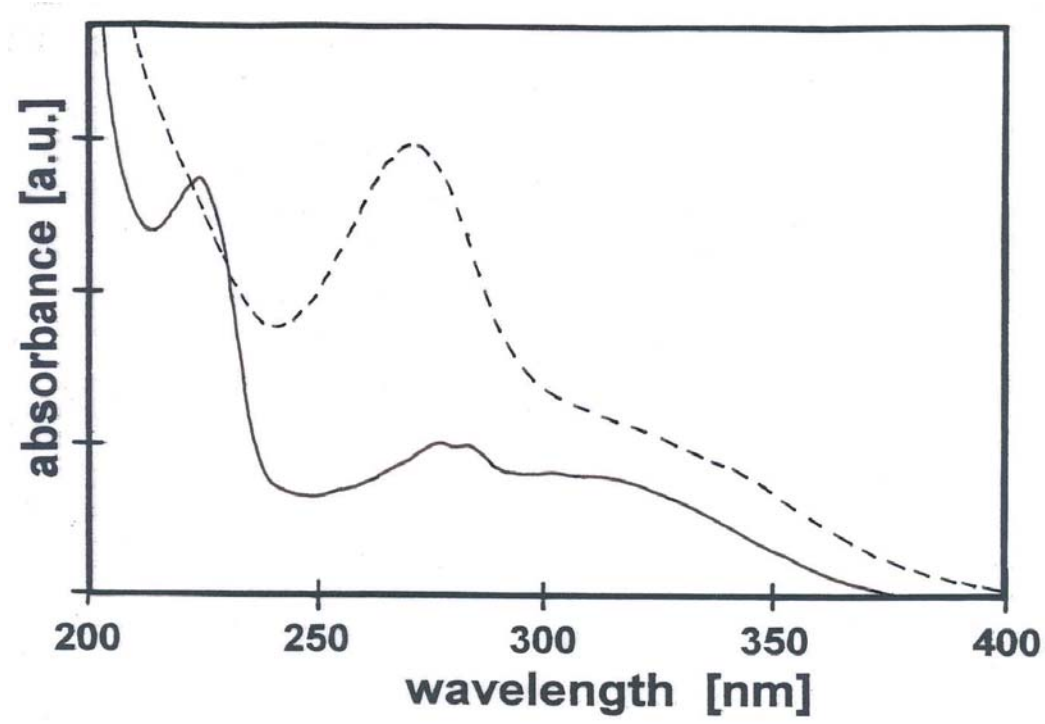


Fig. 50: UV-absorbance spectra of single culture extracts of *Linderina macrospora*. (—) *L. macrospora* pH 2, (---) *L. macrospora* pH 8.

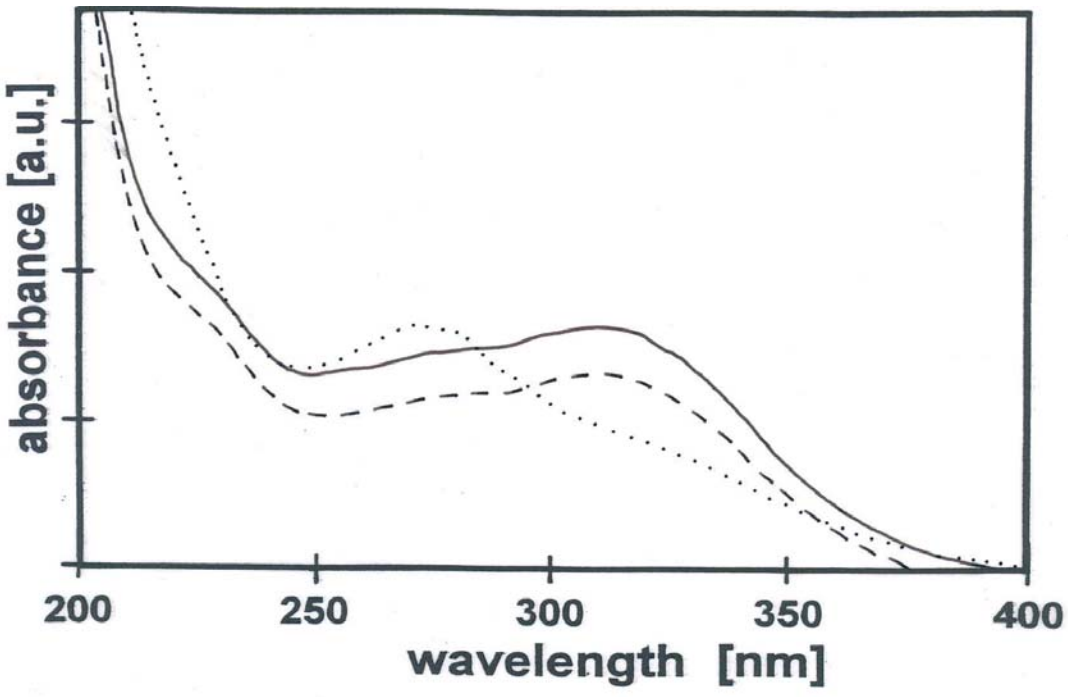


Fig. 51: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Linderina macrospora*. (—) *P. parasitica* (+) x *L. macrospora* pH 2, (---) *P. parasitica* (-) x *L. macrospora* pH 2, (....) *P. parasitica* (-) x *L. macrospora* pH 8.

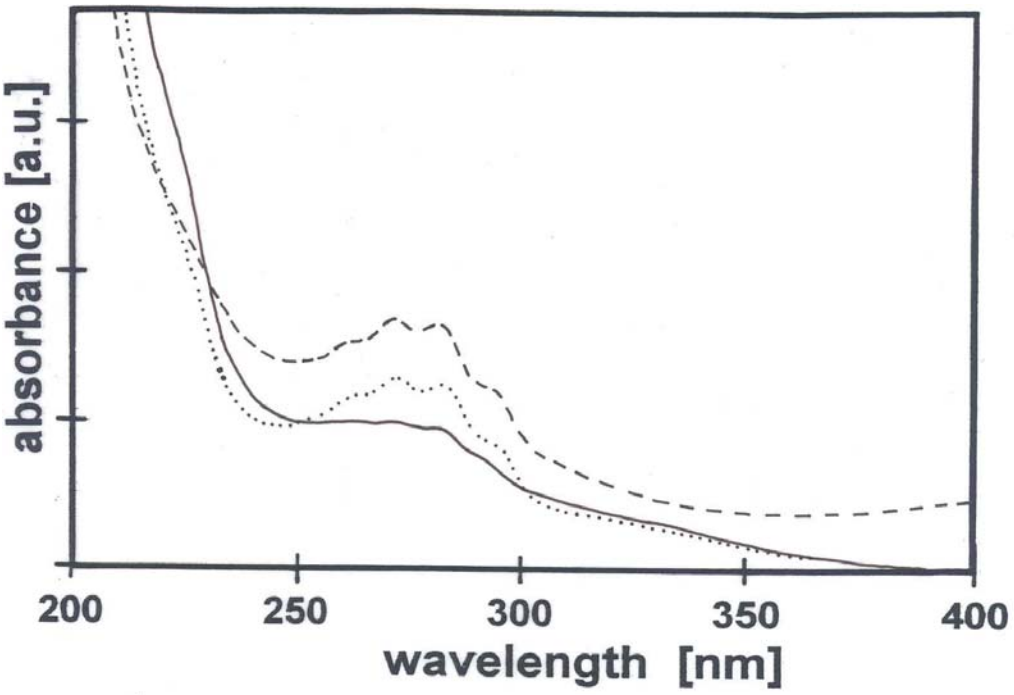


Fig. 52: UV-absorbance spectra of single culture extracts of *Mucor mucedo*.
(—) *M. mucedo* (+) pH 8, (---) *M. mucedo* (-) pH 2, (....) *M. mucedo* (-) pH 8.

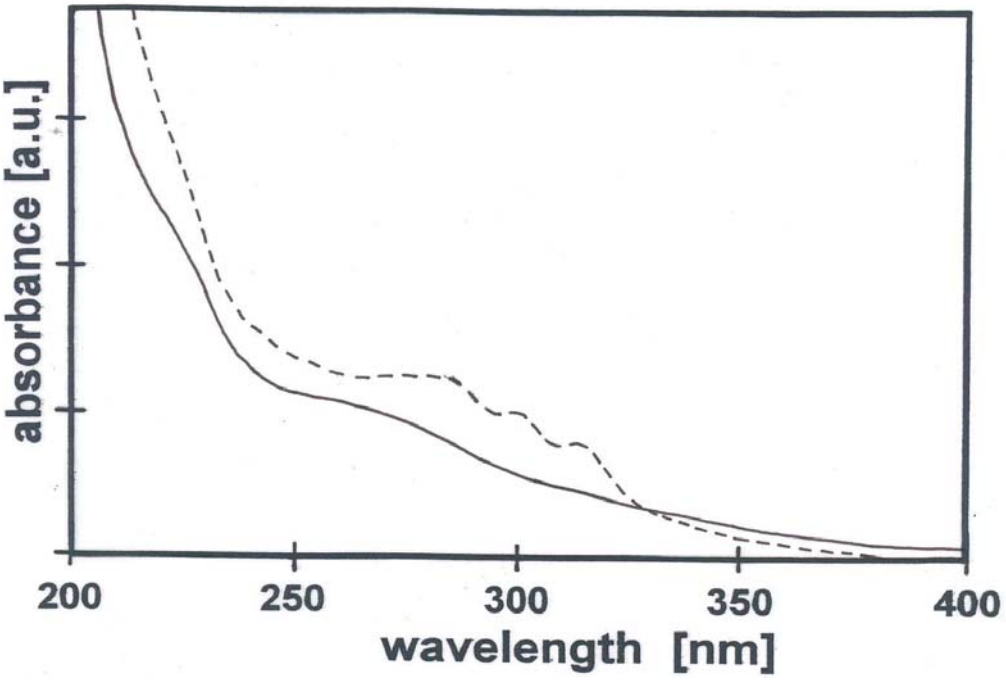


Fig. 53: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Mucor mucedo*. (—) *P. parasitica* (+) x *M. mucedo* (+) pH 8,
(---) *P. parasitica* (+) x *M. mucedo* (+) pH 2.

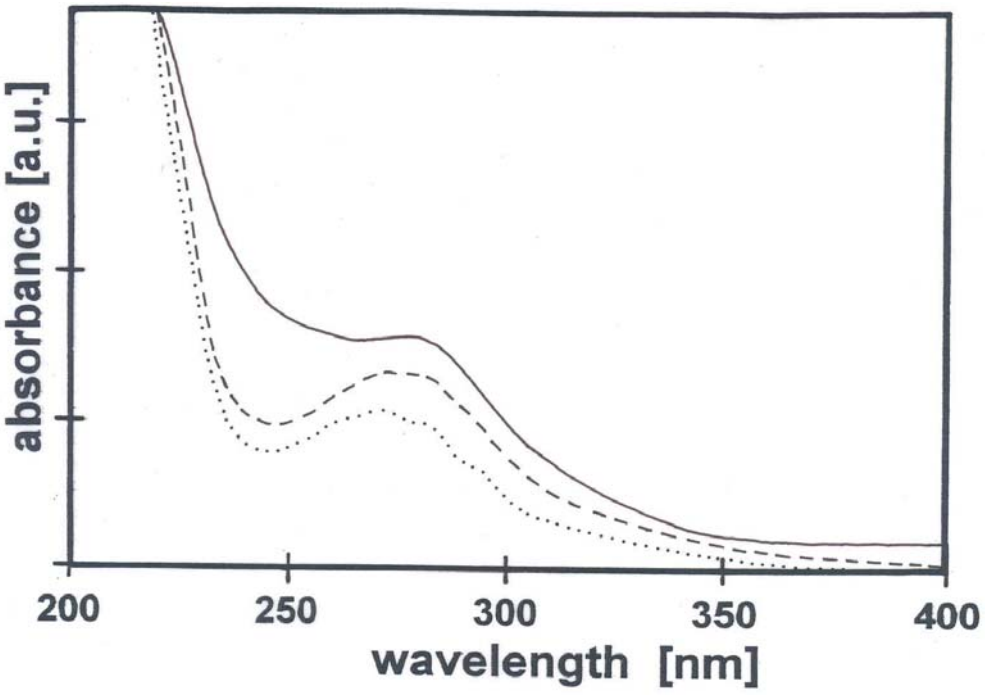


Fig. 54: UV-absorbance spectra of single culture extracts of *Mucor racemosus*.
(—) *M. racemosus* (+) pH 2, (---) *M. racemosus* (+) pH 8,
(....) *M. racemosus* (-) pH 8.

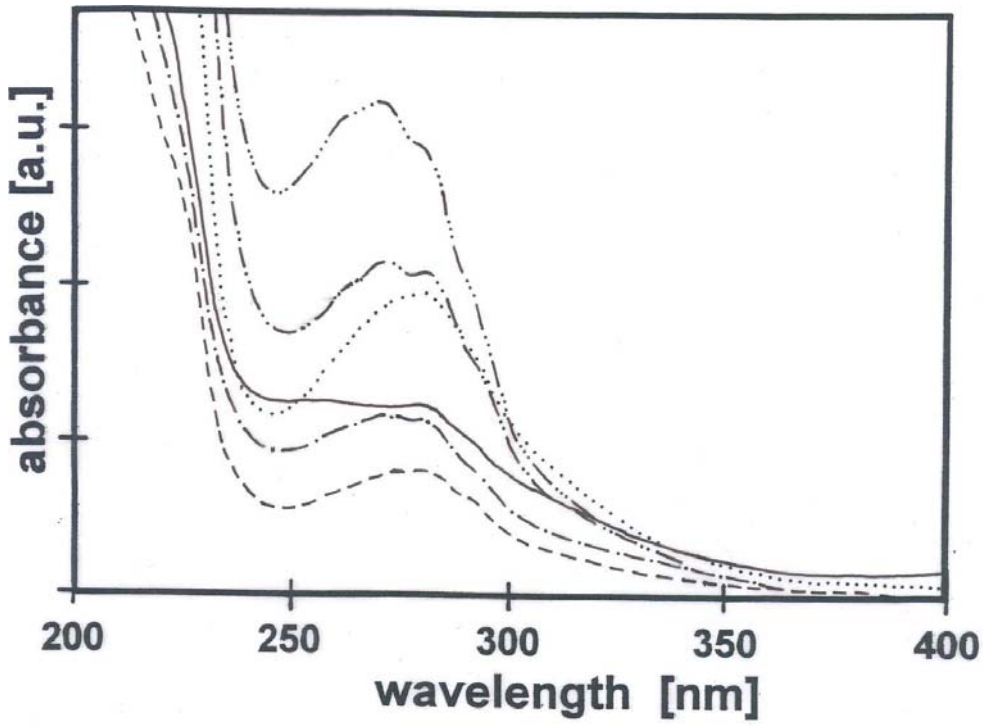


Fig. 55: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Mucor racemosus*. (—) *P. parasitica* (+) x *M. racemosus* (+) pH 2, (---) *P. parasitica* (+) x *M. racemosus* (+) pH 8, (....) *P. parasitica* (-) x *M. racemosus* (+) pH 8, (-.-.-) *P. parasitica* (+) x *M. racemosus* (-) pH 8, (-..-..-) *P. parasitica* (-) x *M. racemosus* (-) pH 8, (-...-...-) *M. racemosus* (+) x *M. racemosus* (-) pH 8.

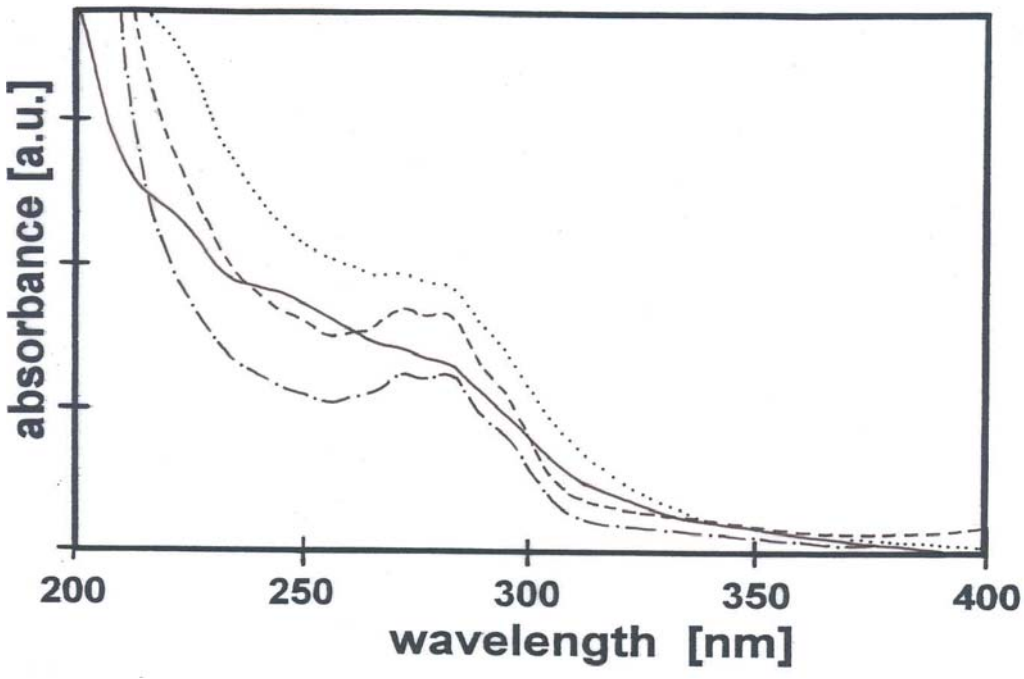


Fig. 56: UV-absorbance spectra of single culture extracts of *Phycomyces blakesleeanus*. (—) *P. blakesleeanus* (+) pH 2, (---) *P. blakesleeanus* (+) pH 8, (....) *P. blakesleeanus* (-) pH 2, (-.-.-) *P. blakesleeanus* (-) pH 8.

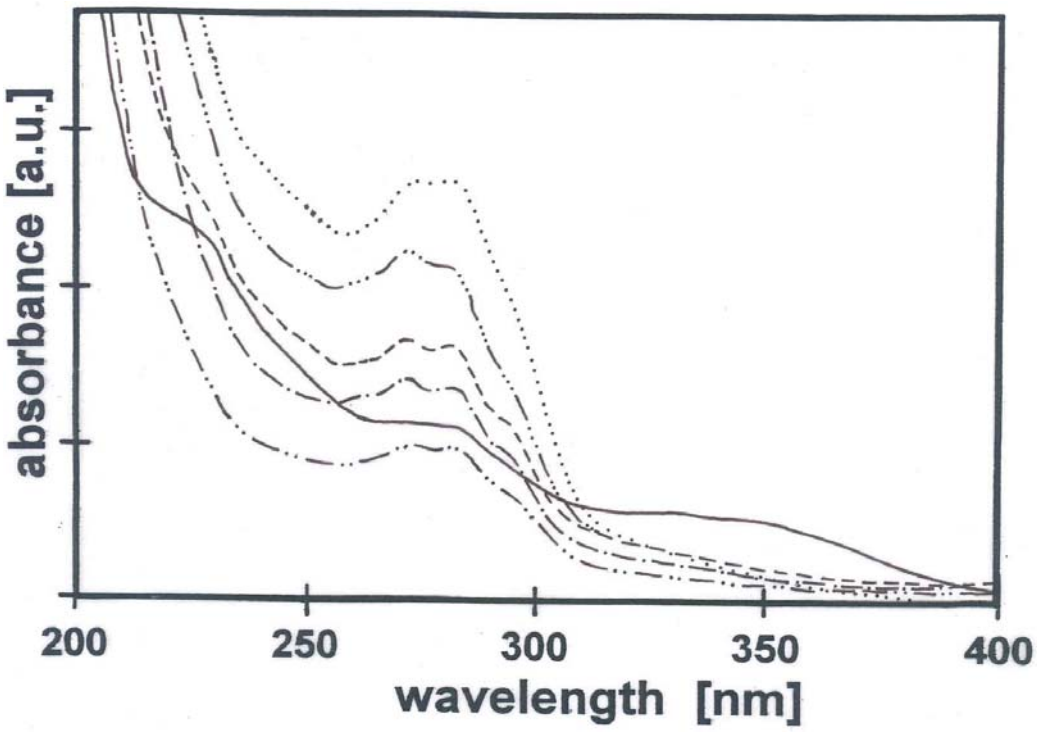


Fig. 57: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Phycomyces blakesleeanus*. (—) *P. parasitica* (+) x *P. blakesleeanus* (+) pH 2, (---) *P. parasitica* (+) x *P. blakesleeanus* (+) pH 8, (....) *P. parasitica* (-) x *P. blakesleeanus* (+) pH 8, (-.-.-) *P. parasitica* (+) x *P. blakesleeanus* (-) pH 8, (-..-..-) *P. parasitica* (-) x *P. blakesleeanus* (-) pH 8, (-...-...-) *P. blakesleeanus* (+) x *P. blakesleeanus* (-) pH 8.

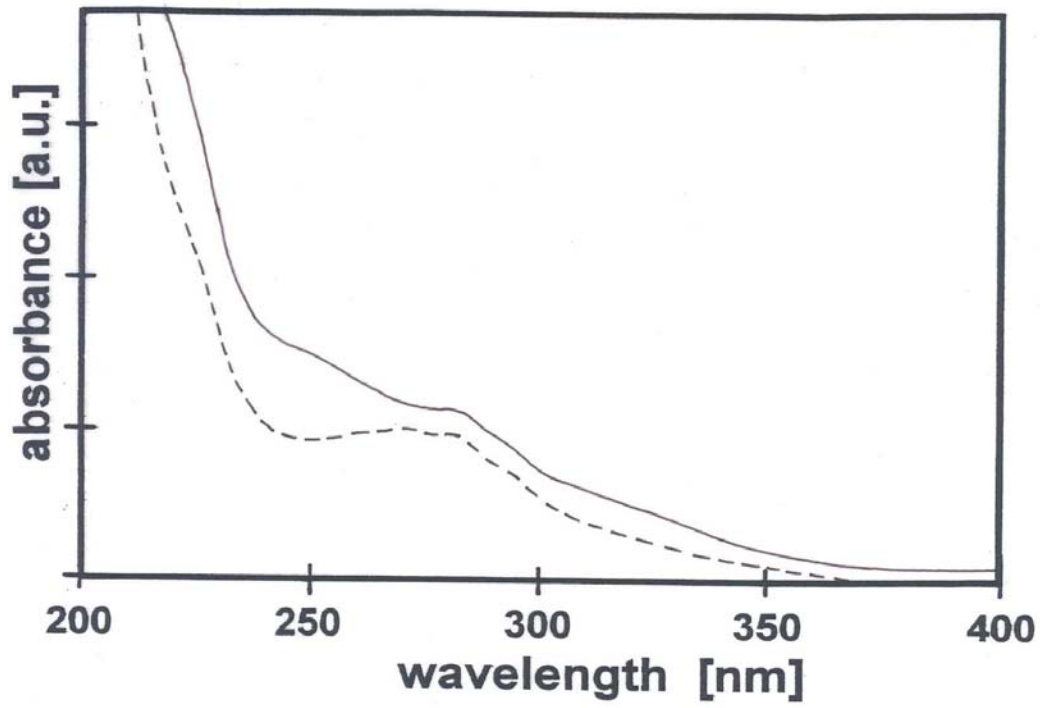


Fig. 58: UV-absorbance spectra of single culture extracts of *Pilaira anomala*. (—) *P. anomala* pH 2, (---) *P. anomala* pH 8.

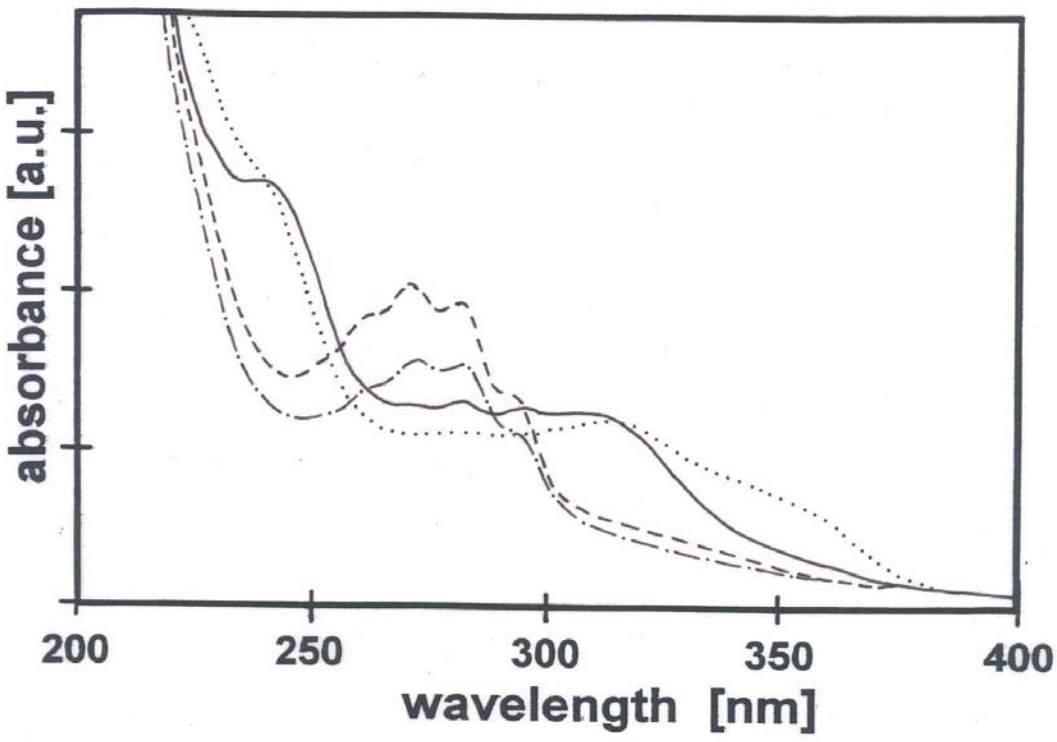


Fig. 59: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Pilaira anomala*. (—) *P. parasitica* (+) x *P. anomala* pH 2, (---) *P. parasitica* (+) x *P. anomala* pH 8, (....) *P. parasitica* (-) x *P. anomala* pH 2, (-.-.-) *P. parasitica* (-) x *P. anomala* pH 8.

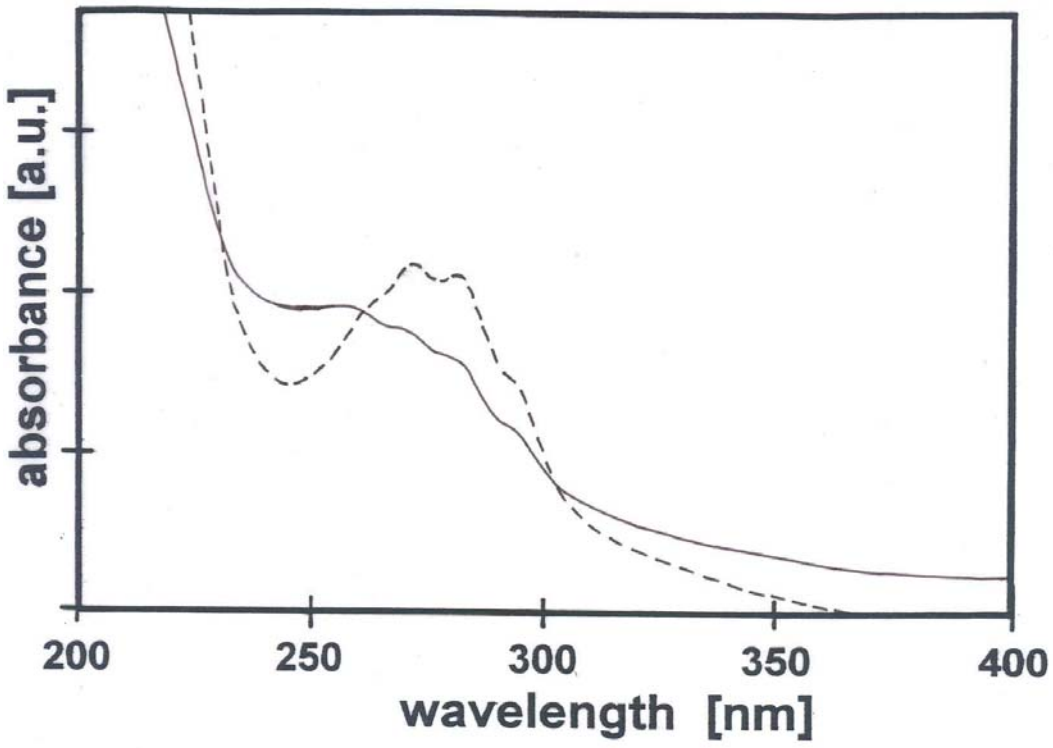


Fig. 60: UV-absorbance spectra of single culture extracts of *Syzigites megalocarpus*. (—) *S. megalocarpus* pH 2, (---) *S. megalocarpus* pH 8.

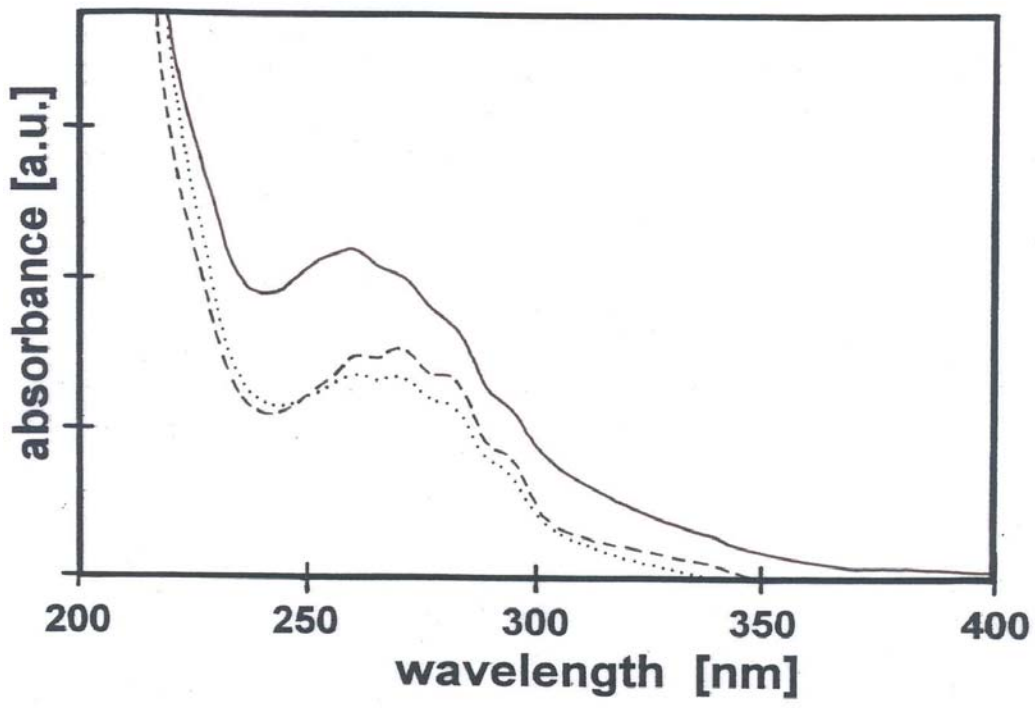


Fig. 61: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* and *Syzigites megalocarpus*. (—) *P. parasitica* (+) x *S. megalocarpus* pH 2, (---) *P. parasitica* (+) x *S. megalocarpus* pH 8, (....) *P. parasitica* (-) x *S. megalocarpus* pH 8.

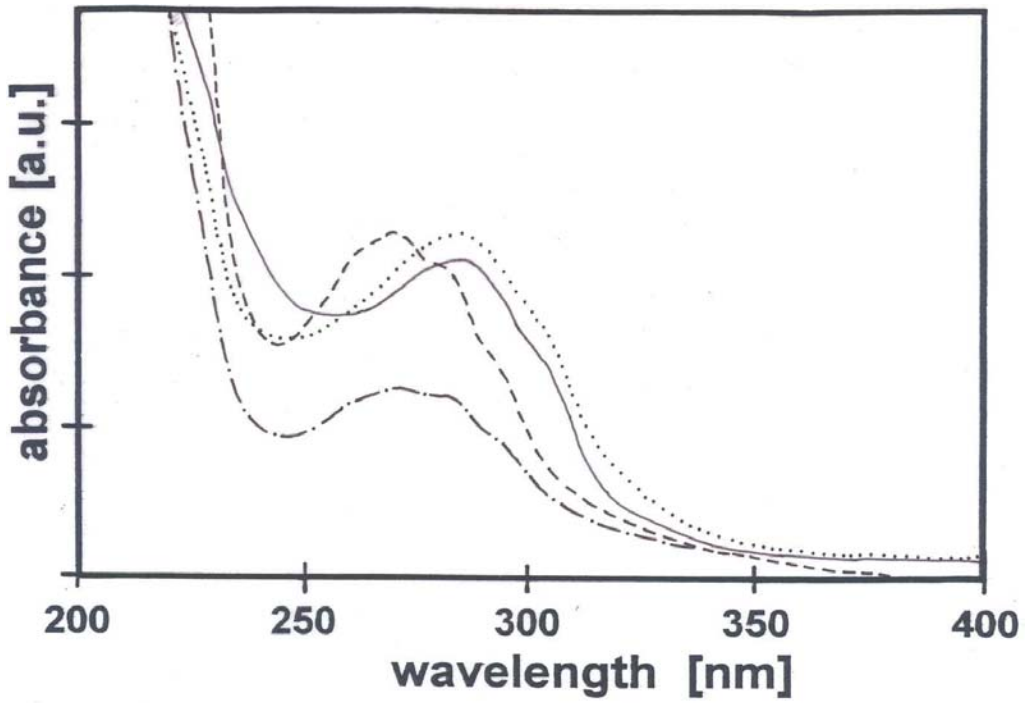


Fig. 62: UV-absorbance spectra of single culture extracts of *Thamnidium elegans*. (—) *T. elegans* (+) pH 2, (---) *T. elegans* (+) pH 8, (....) *T. elegans* (-) pH 2, (-.-.-) *T. elegans* (-) pH 8.

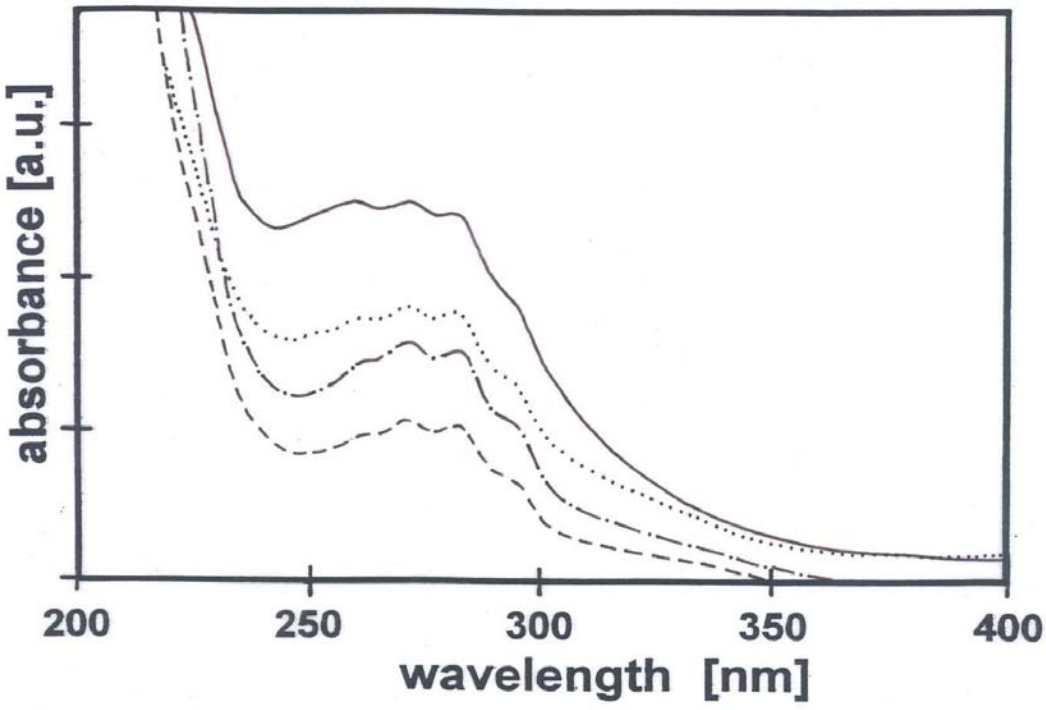


Fig. 63: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* (+) and *Thamnidium elegans*. (—) *P. parasitica* (+) x *T. elegans* (+) pH 2, (---) *P. parasitica* (+) x *T. elegans* (+) pH 8, (....) *P. parasitica* (+) x *T. elegans* (-) pH 2, (-.-.-) *P. parasitica* (+) x *T. elegans* (-) pH 8.

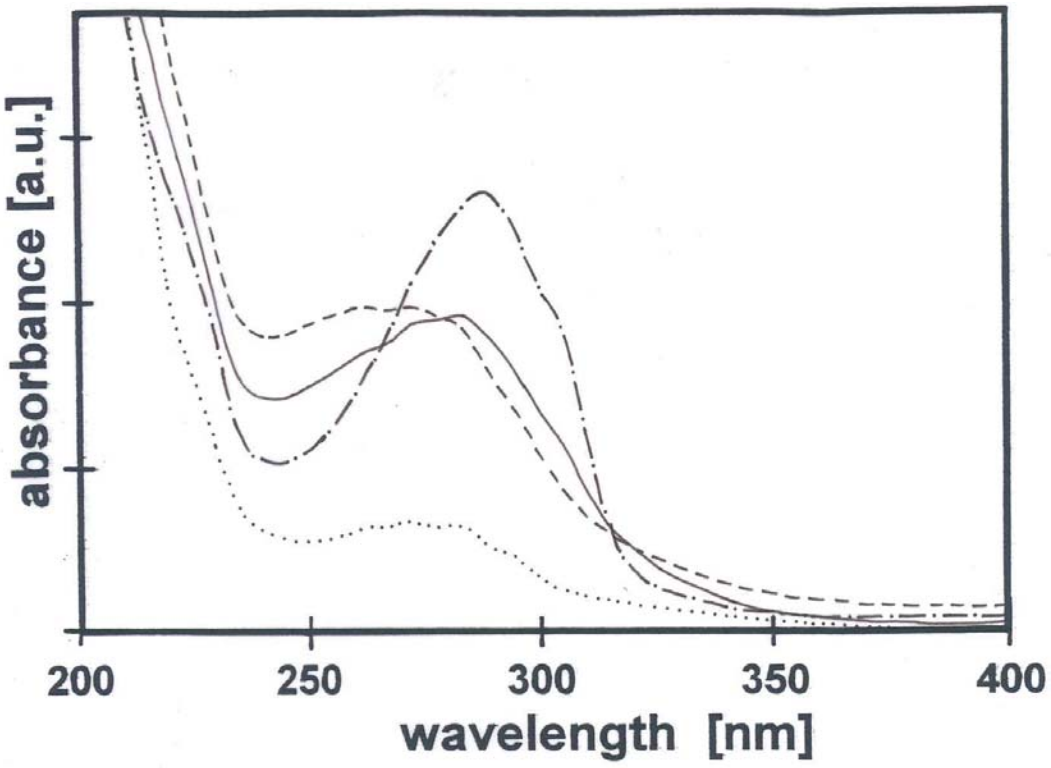


Fig. 64: UV-absorbance spectra of combined culture extracts of *Parasitella parasitica* (-) and *Thamnidium elegans*. (—) *P. parasitica* (-) x *T. elegans* (+) pH 2, (---) *P. parasitica* (-) x *T. elegans* (-) pH 2, (....) *P. parasitica* (-) x *T. elegans* (-) pH 8, (-.-.-) *T. elegans* (+) x *T. elegans* (-) pH 2.

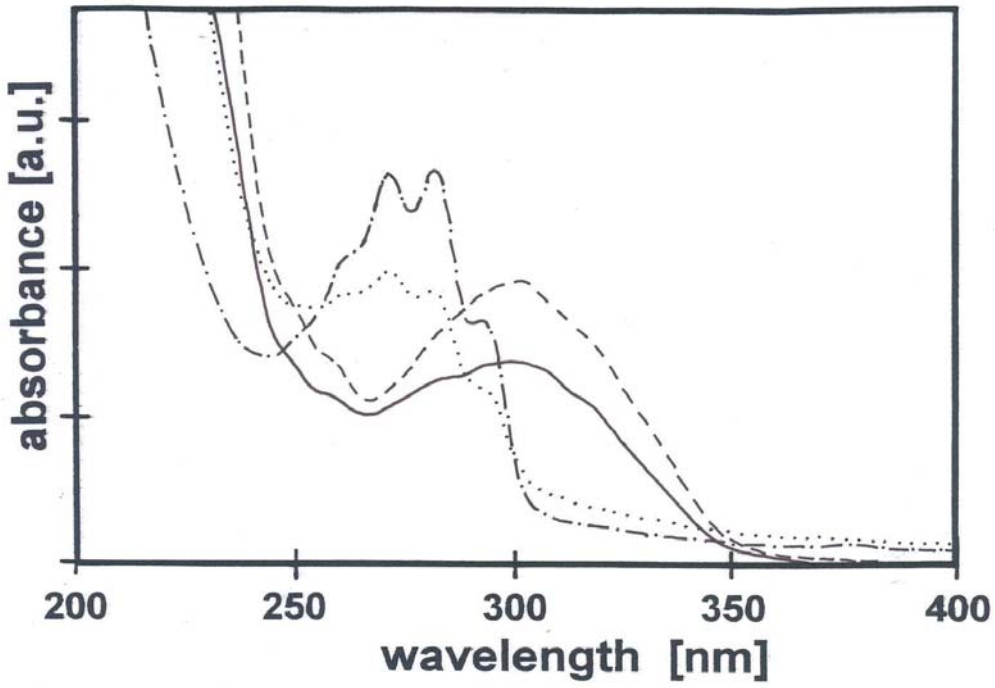


Fig. 65: UV-absorbance spectra of tlc single bands obtained in the single culture extract of *P. parasitica* (-) and in the combined culture extracts of (+) and (-) mating types of *P. parasitica*. (—) *P. parasitica* (-) pH 2, (---) *P. parasitica* (-) pH 8, (....) *P. parasitica* (+) x *P. parasitica* (-) pH 2, (-.-.-) *P. parasitica* (+) x *P. parasitica* (-) pH 8.

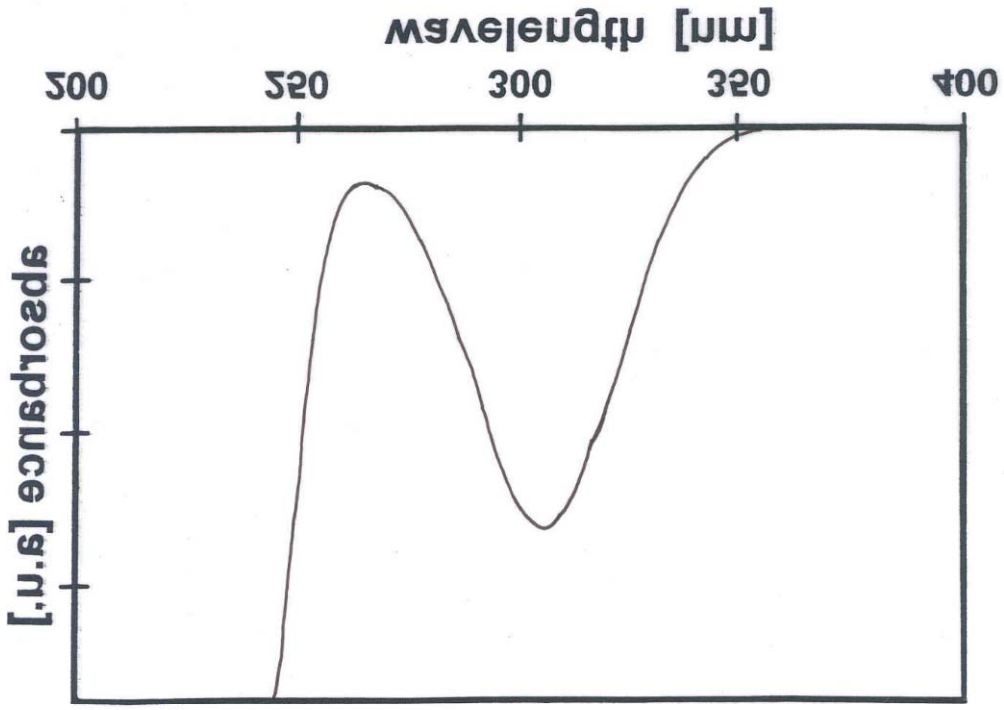


Fig. 66: UV-absorbance spectra of tlc single band obtained in the pH 2 combined culture extract of *P. parasitica* (+) and *Chaetocladium brefeldii*.

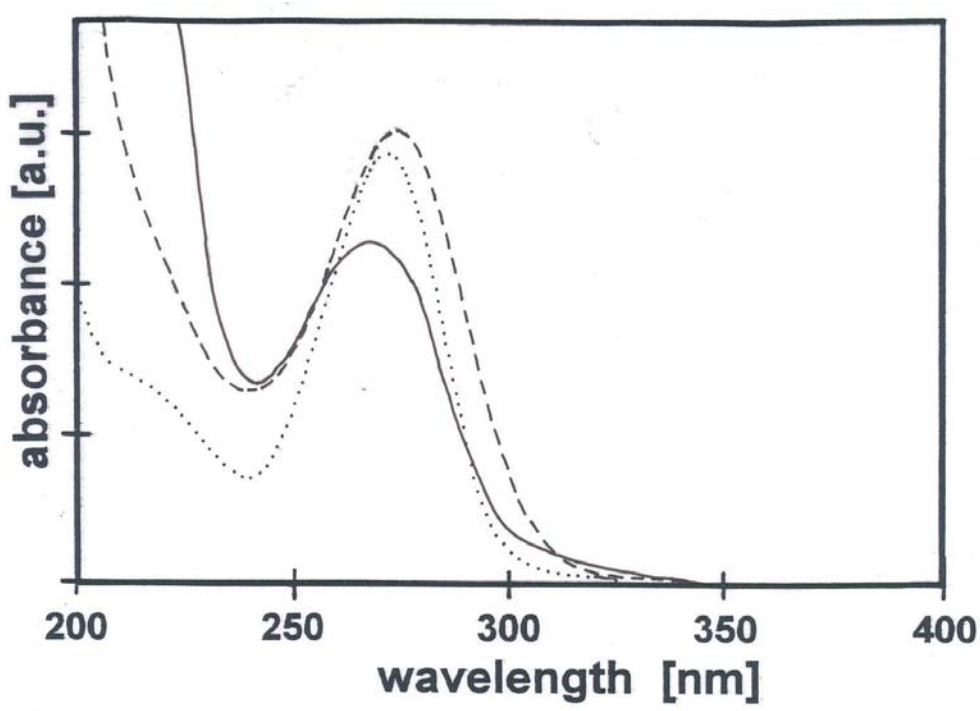


Fig. 67: UV-absorbance spectra of tlc single bands obtained in the pH 2 and pH 8 single culture extracts of *Coemansia formosensis*. (—) band in pH 2 extract, (---) band in pH 8, migrating fast on silica gel, (....) band in pH 8, migrating slower.

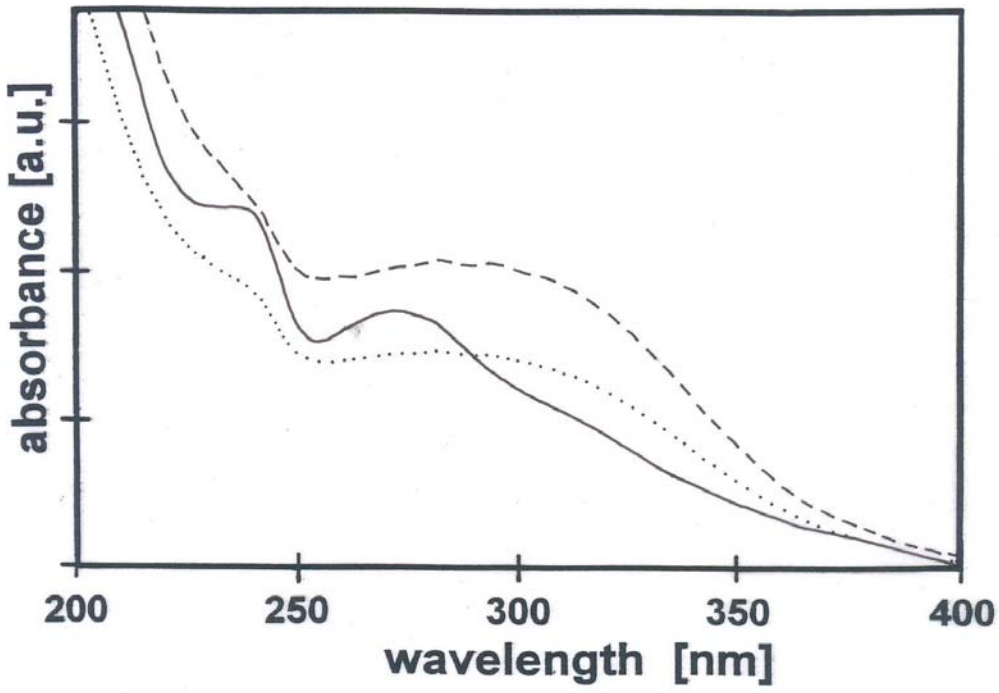


Fig. 68: UV-absorbance spectra of tlc single bands obtained in the pH 2 single culture extract of *Linderina macrospora* (—) and in the pH 2 combined culture extract of *P. parasitica* (+) x *L. macrospora* (---) and *P. parasitica* (-) x *L. macrospora* (....).

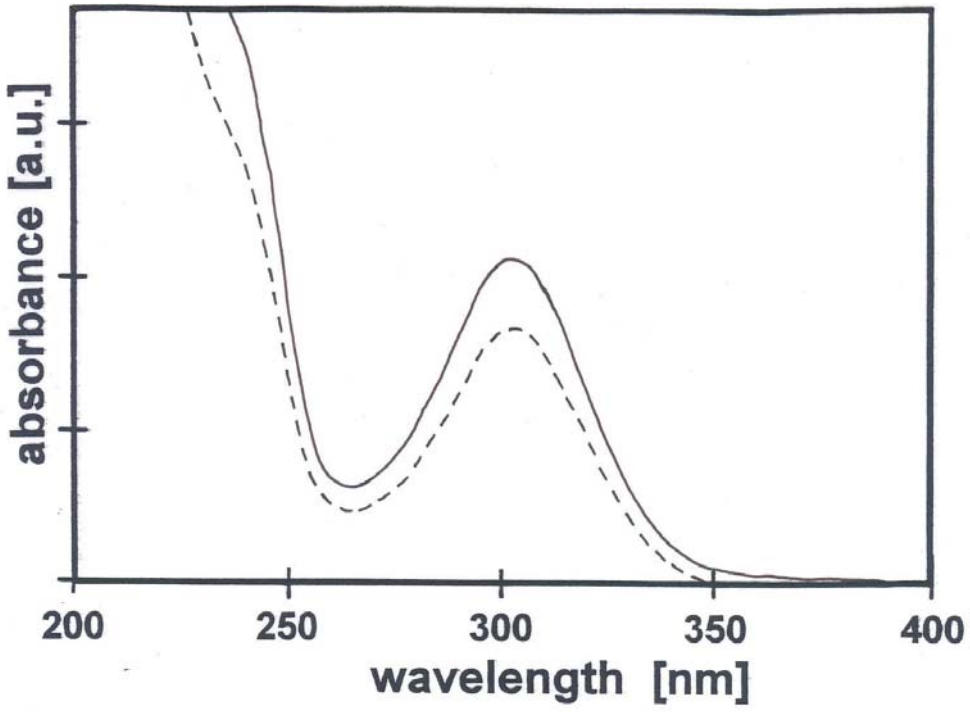


Fig. 69: UV-absorbance spectra of tlc single bands obtained in the pH 2 combined culture extract of *P. parasitica* (+) x *Pilaira anomala* (—) and *P. parasitica* (-) x *P. anomala* (---).

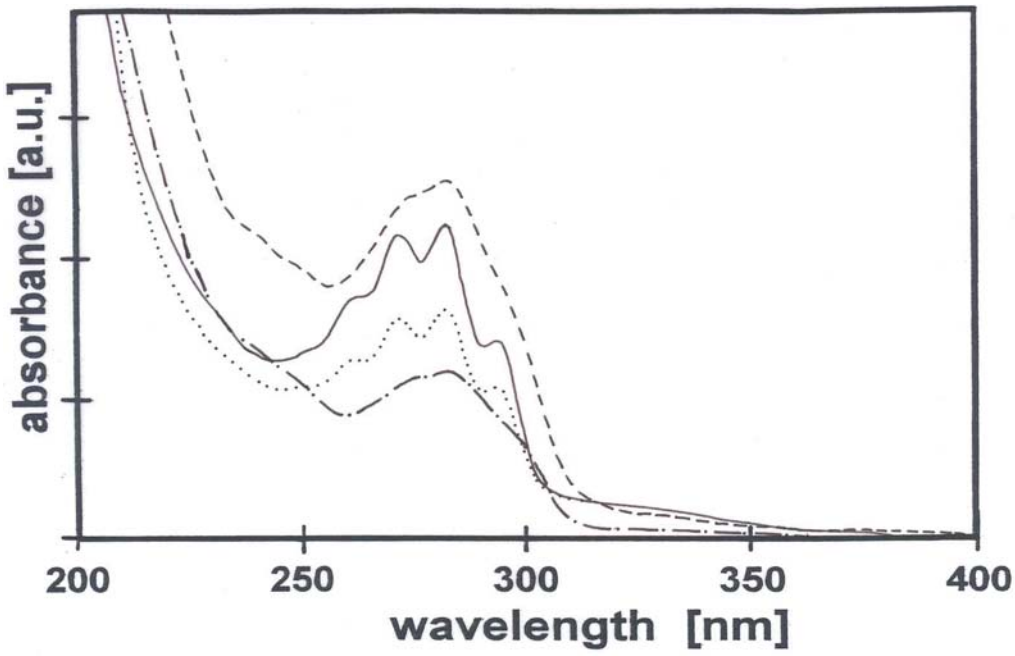


Fig. 70: UV-absorbance spectra of tlc single bands obtained in the pH 8 single culture extract of *Phycomyces blakesleeanus*. (—) band of *P. blakesleeanus* (+), migrating fast on silica gel, (---) band of *P. blakesleeanus* (+), migrating slower, (....) band of *P. blakesleeanus* (-), migrating fast, (-.-.-) band of *P. blakesleeanus* (-), migrating slower.

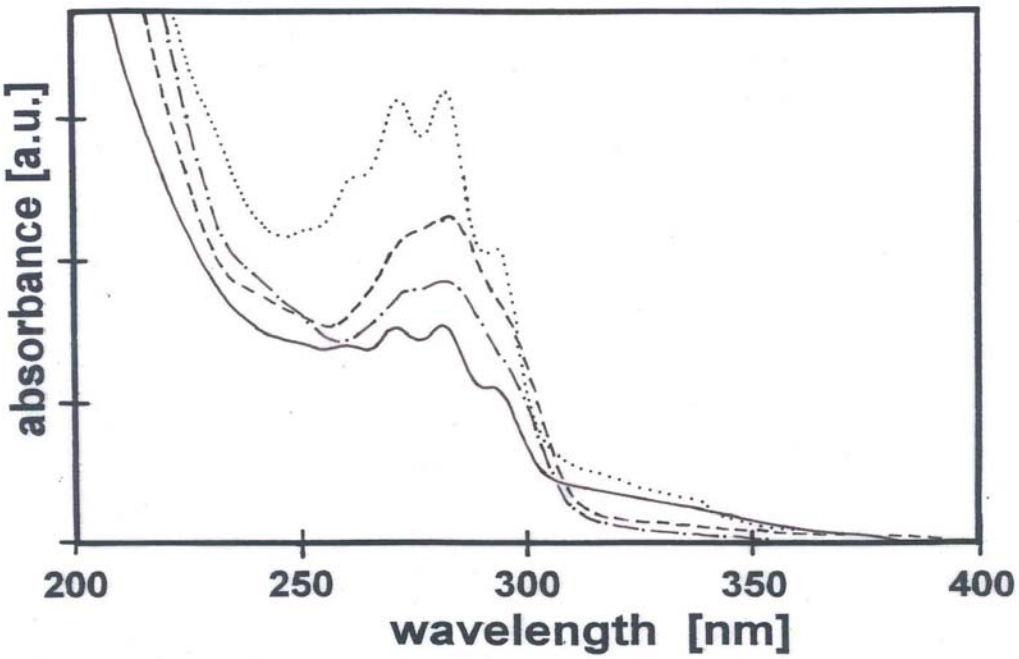


Fig. 71: UV-absorbance spectra of tlc single bands obtained in the pH 8 combined culture extracts of *P. parasitica* and *P. blakesleeanus*. (—) band of *P. parasitica* (-) x *P. blakesleeanus* (+), migrating fast on silica gel, (---) band of *P. parasitica* (-) x *P. blakesleeanus* (+), migrating slower, (....) band of *P. blakesleeanus* (+) x *P. blakesleeanus* (-), migrating fast, (-.-.-) band of *P. blakesleeanus* (+) x *P. blakesleeanus* (-), migrating slower.

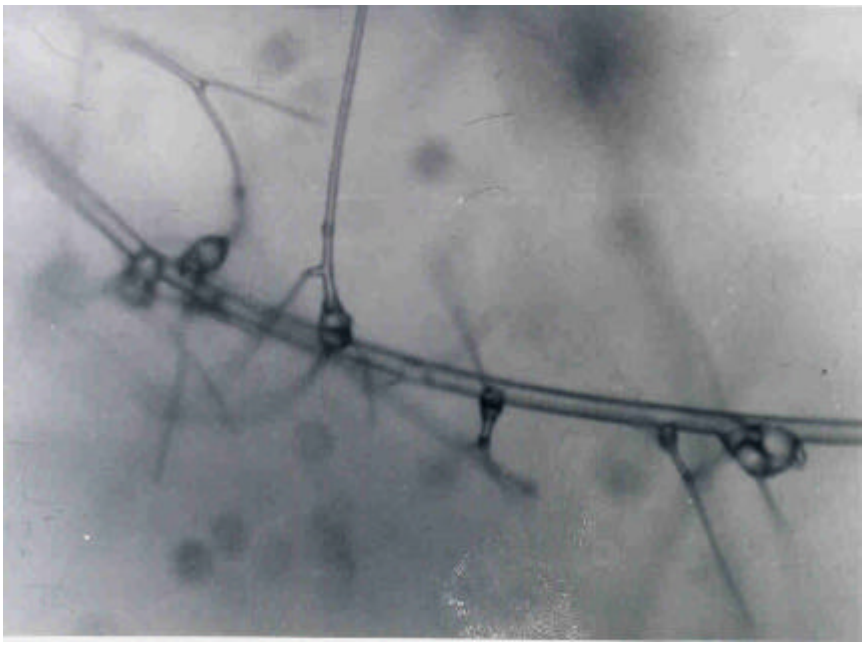


Fig 72: Parasitic interaction between *P. parasitica* (+) and *A. glauca* 101.48 (-)

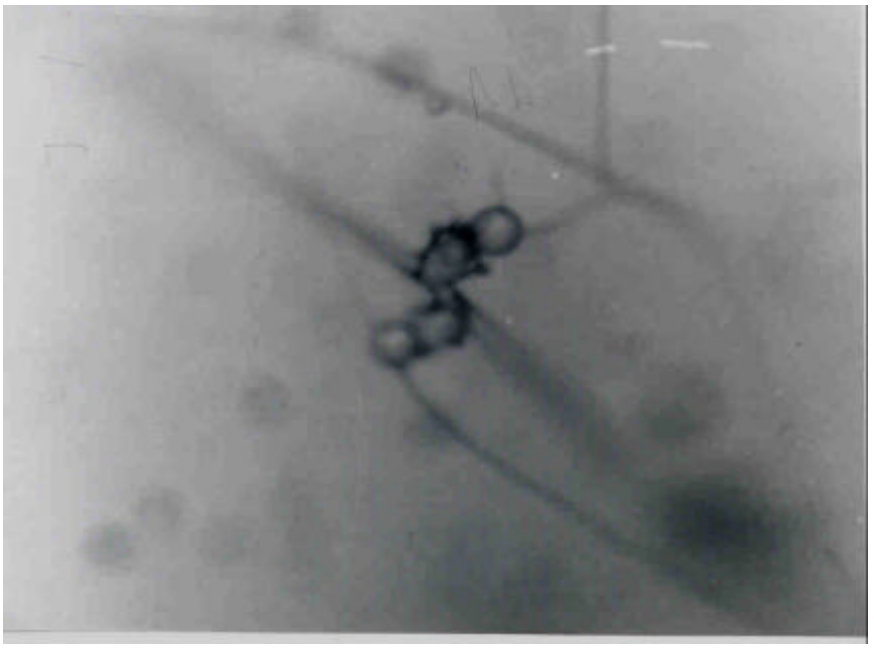


Fig 73: Parasitic interaction between *P. parasitica* (+) and *A. glauca* 101.48 (-)

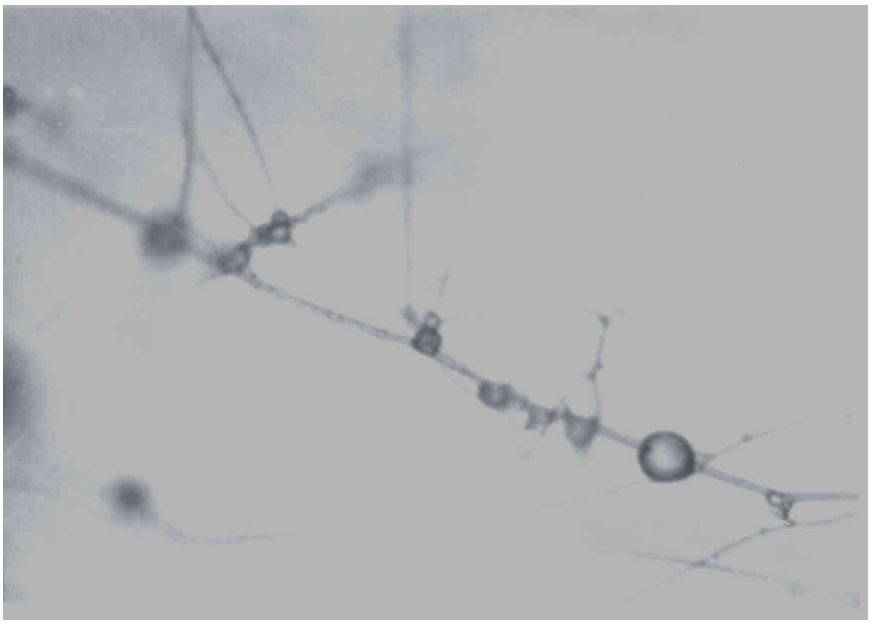


Fig 74: Parasitic interaction between *P. parasitica* (+) and *A. glauca* 6776b (-)

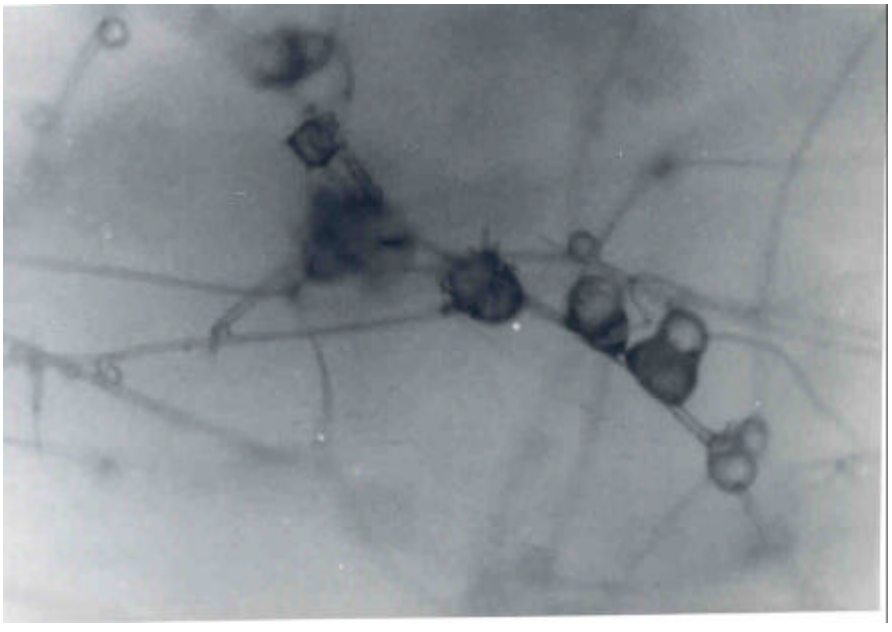


Fig 75: Parasitic interaction between *P. parasitica* (+) and *A. glauca* 6776b (-)

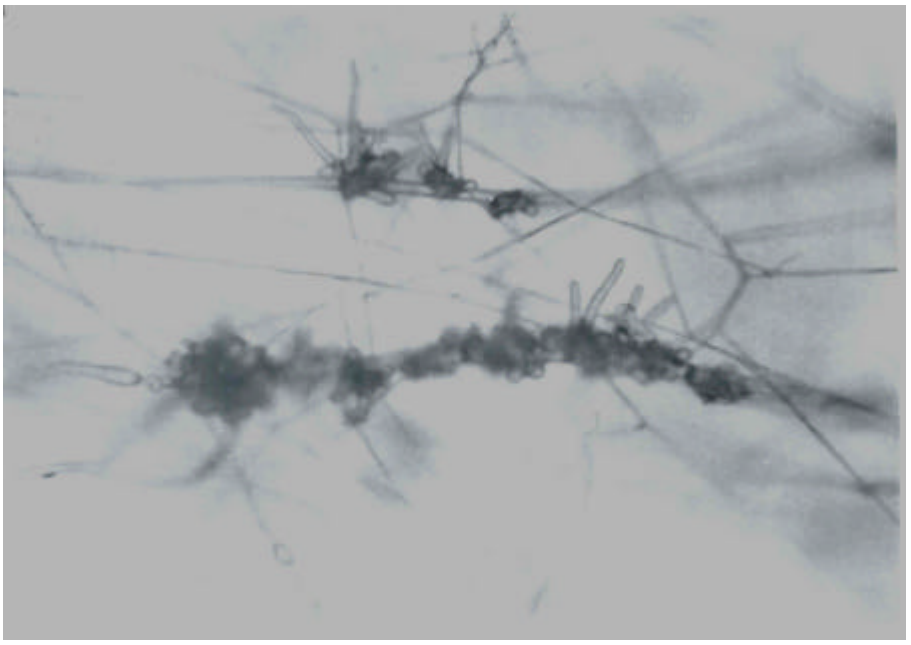


Fig 76: Parasitic interaction between *P. parasitica* (+) and *A. parricida*

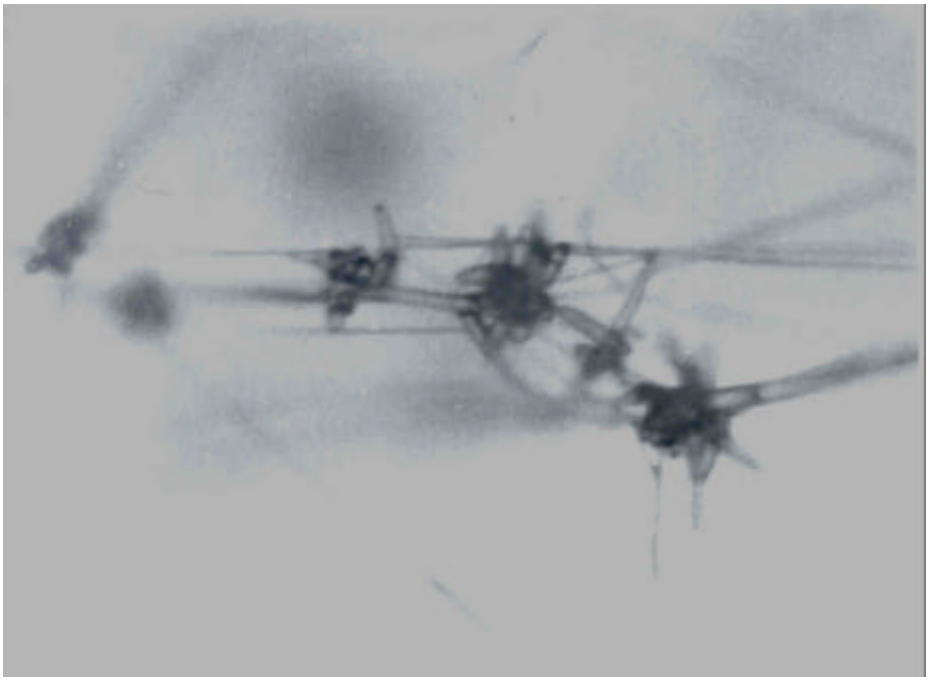


Fig 77: Parasitic interaction between *P. parasitica* (-) and *A. parricida*

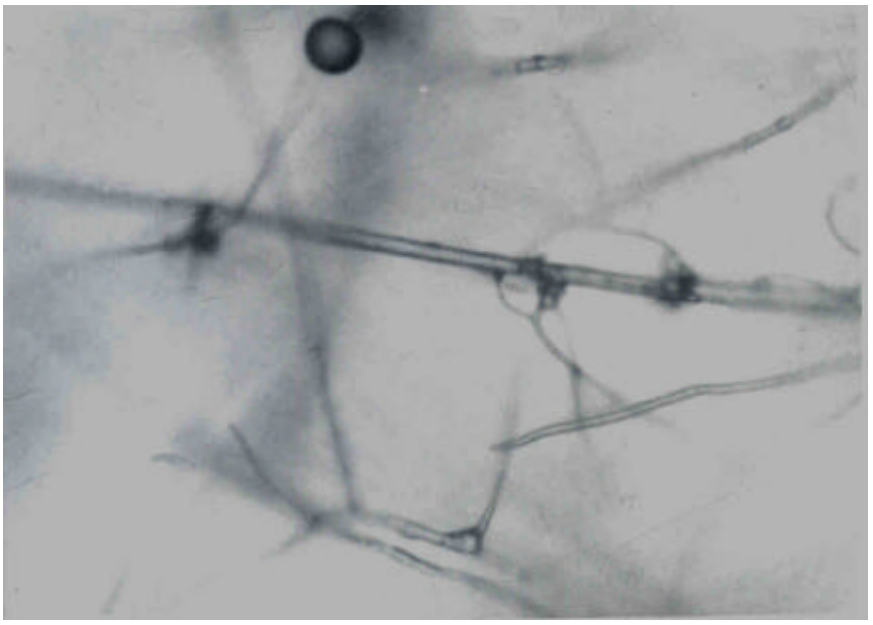


Fig 78: Parasitic interaction between *P. parasitica* (+) and *A. elegans*

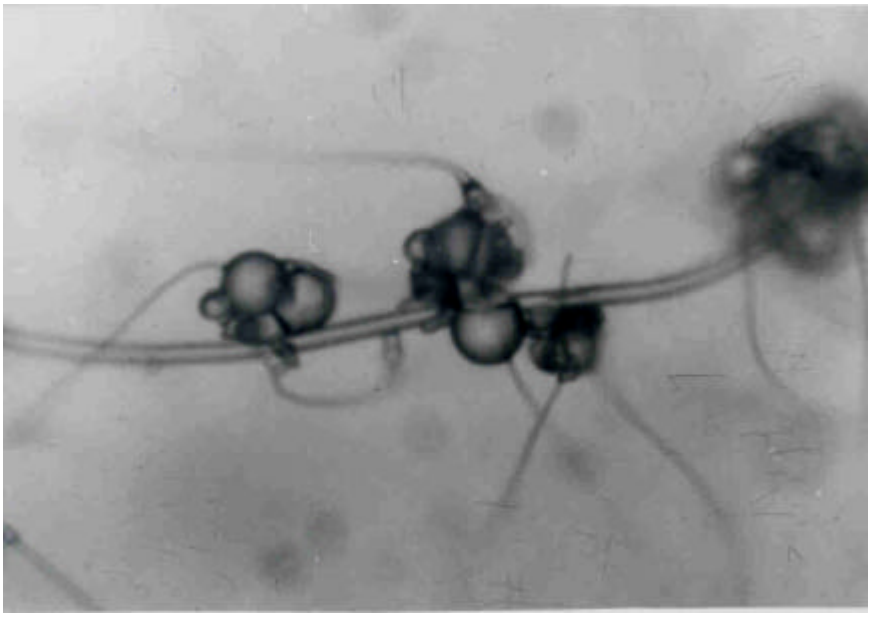


Fig 79: Parasitic interaction between *P. parasitica* (+) and *B. trispora* (-)

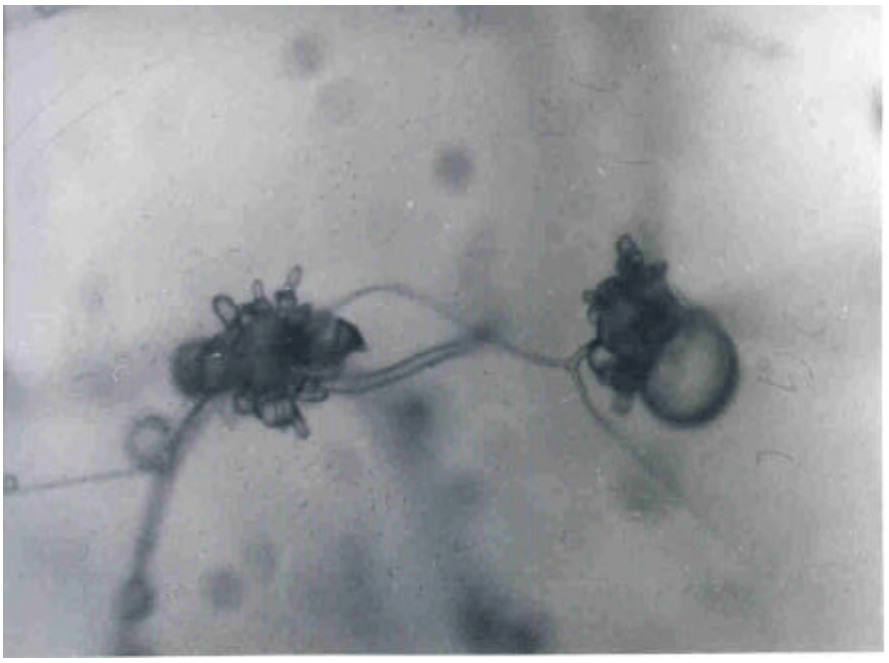


Fig 80: Parasitic interaction between *P. parasitica* (-) and *B. trispora* (-)

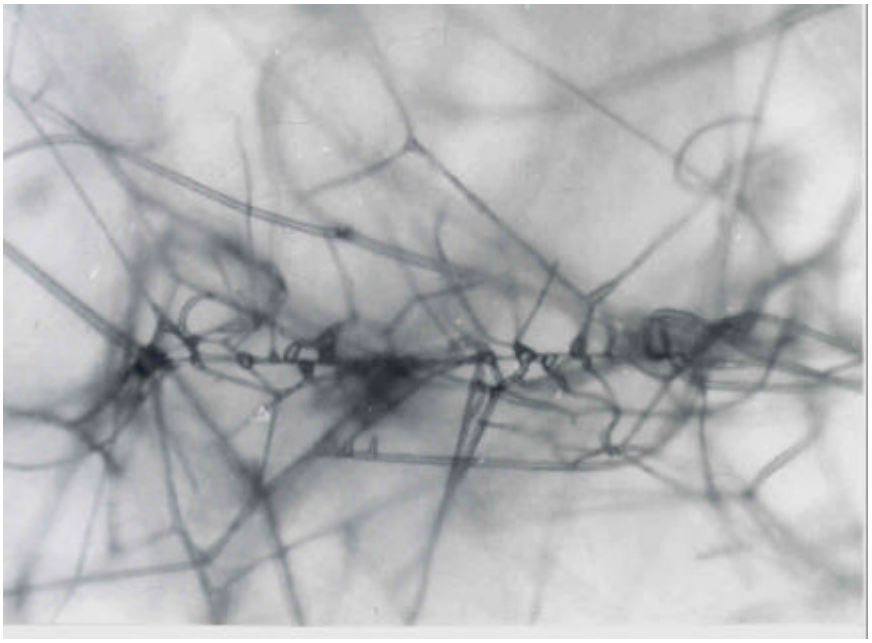


Fig 81: Parasitic interaction between *P. parasitica* (+) and *C. brefeldii*

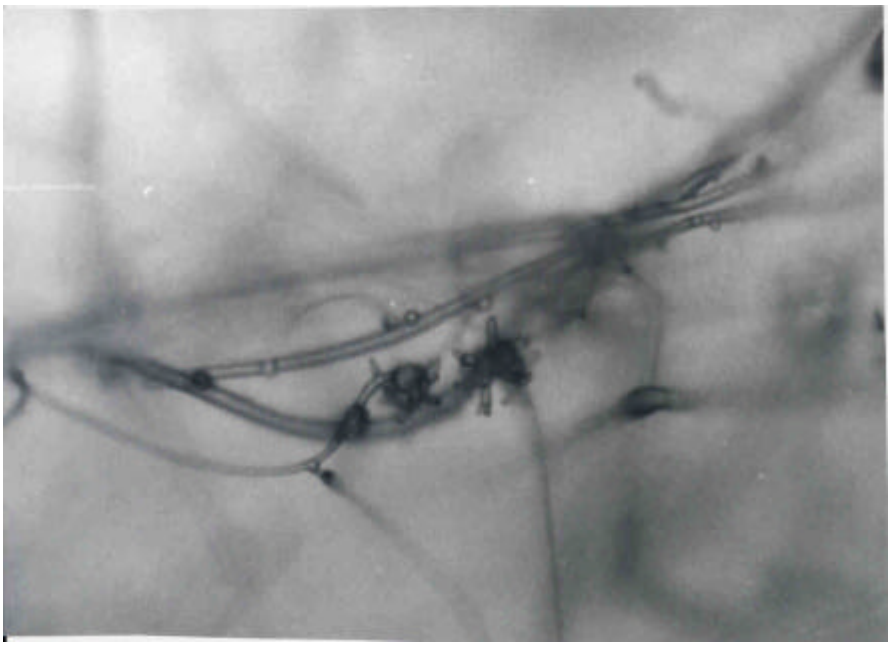


Fig 82: Parasitic interaction between *P. parasitica* (+) and *G. persicaria*

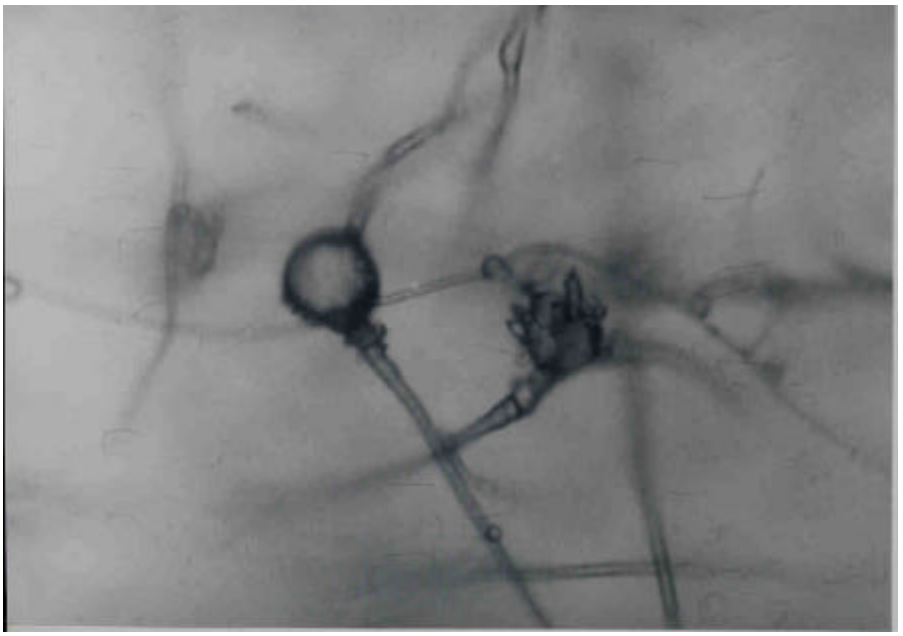


Fig 83: Parasitic interaction between *P. parasitica* (+) and *G. persicaria*

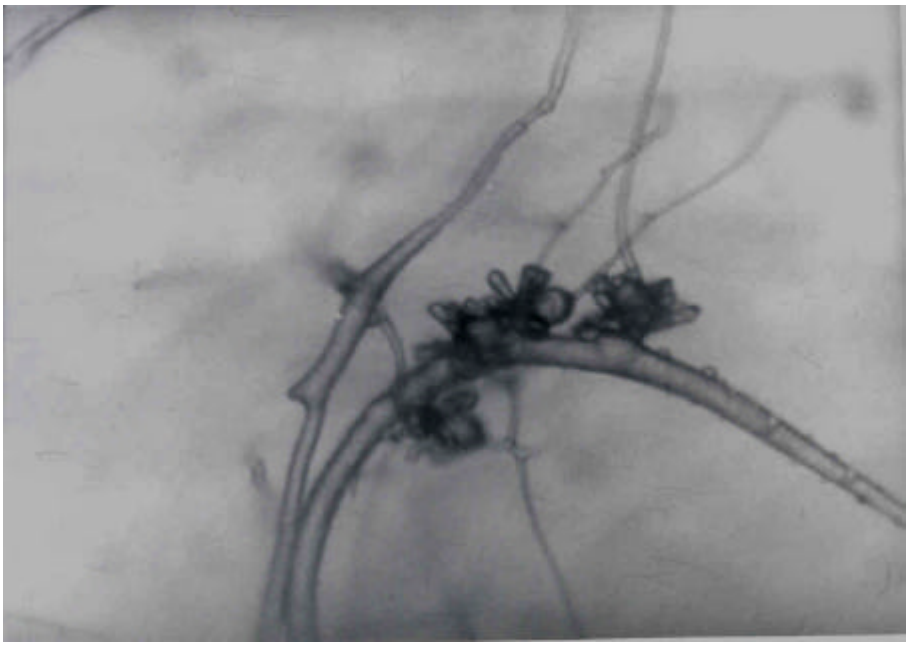


Fig 84: Parasitic interaction between *P. parasitica* (-) and *G. persicaria*

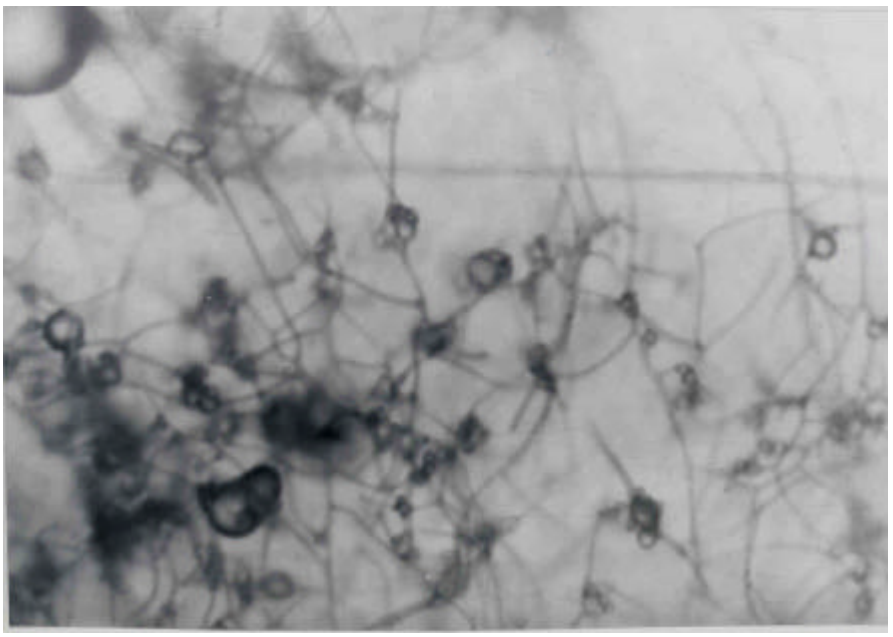


Fig 85: Parasitic interaction between *P. parasitica* (-) and *L. macrospora*

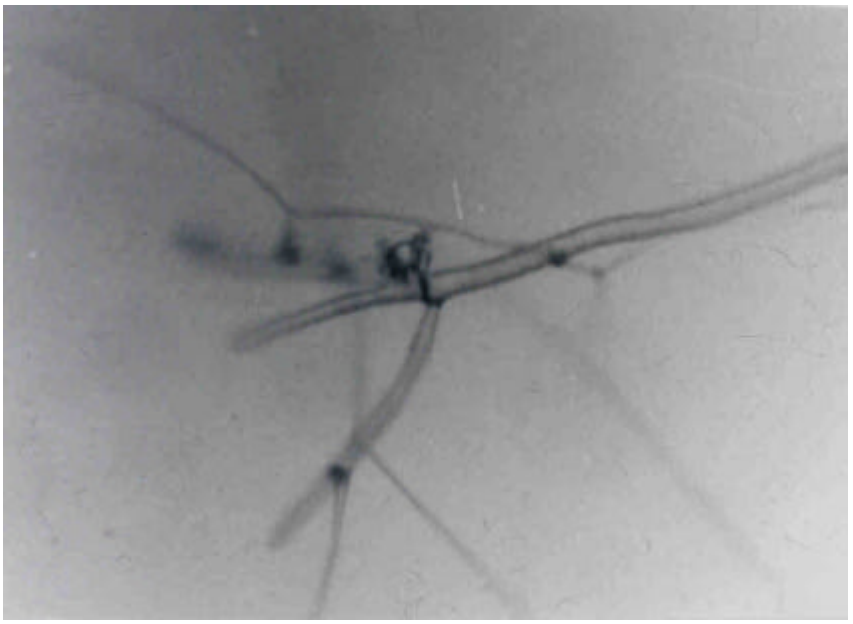


Fig 86: Parasitic interaction between *P. parasitica* (-) and *L. macrospora*

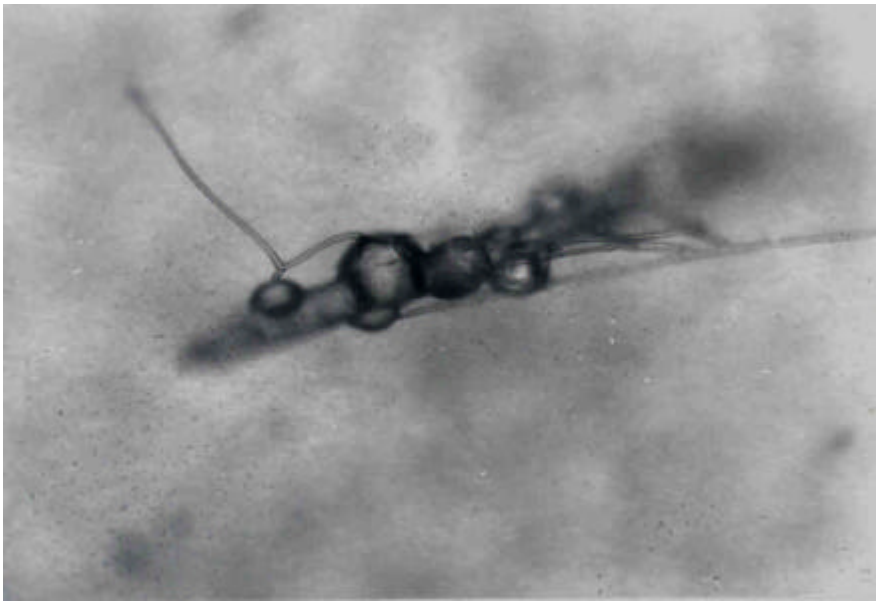


Fig 87: Parasitic interaction between *P. parasitica* (+) and *M. mucedo* (+)

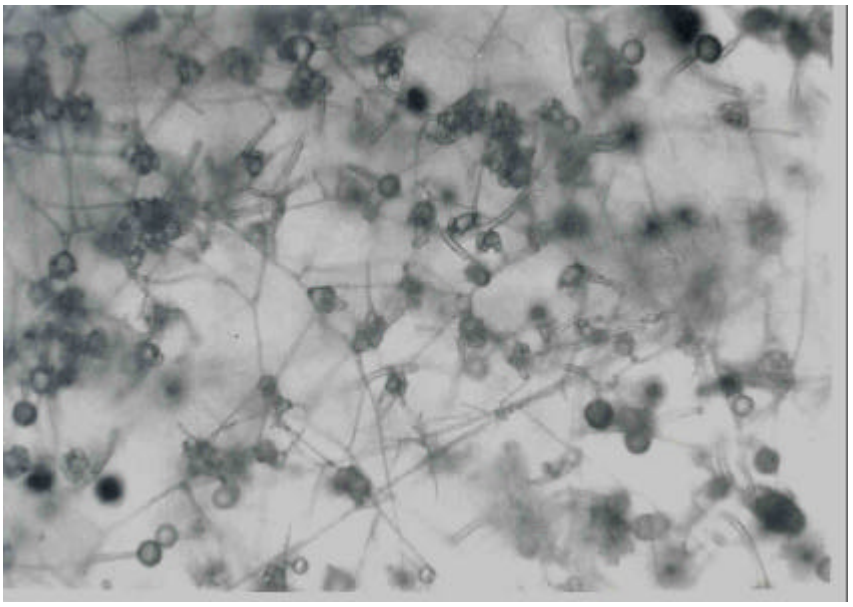


Fig 88: Parasitic interaction between *P. parasitica* (-) and *M. mucedo* (+)

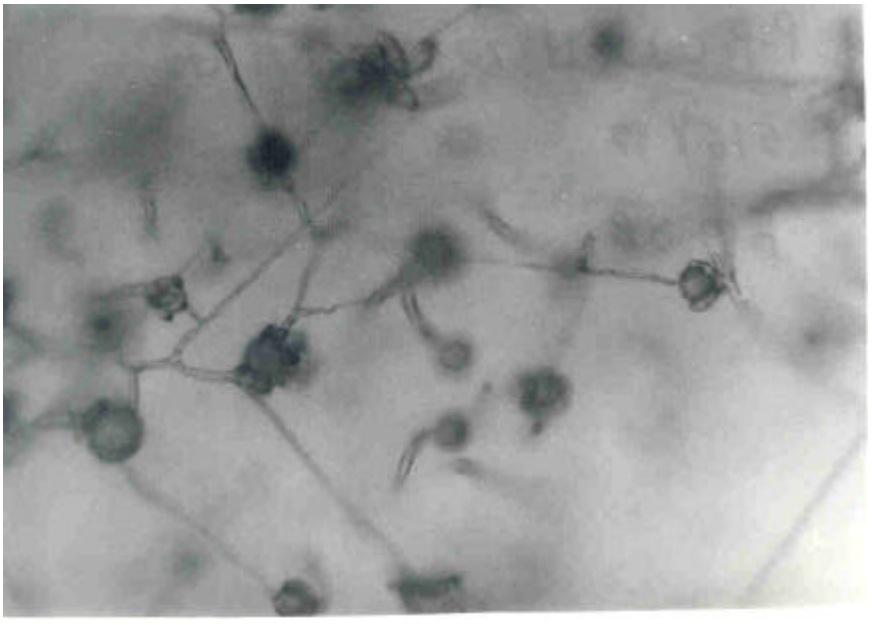


Fig 89: Parasitic interaction between *P. parasitica* (-) and *M. mucedo* (+)

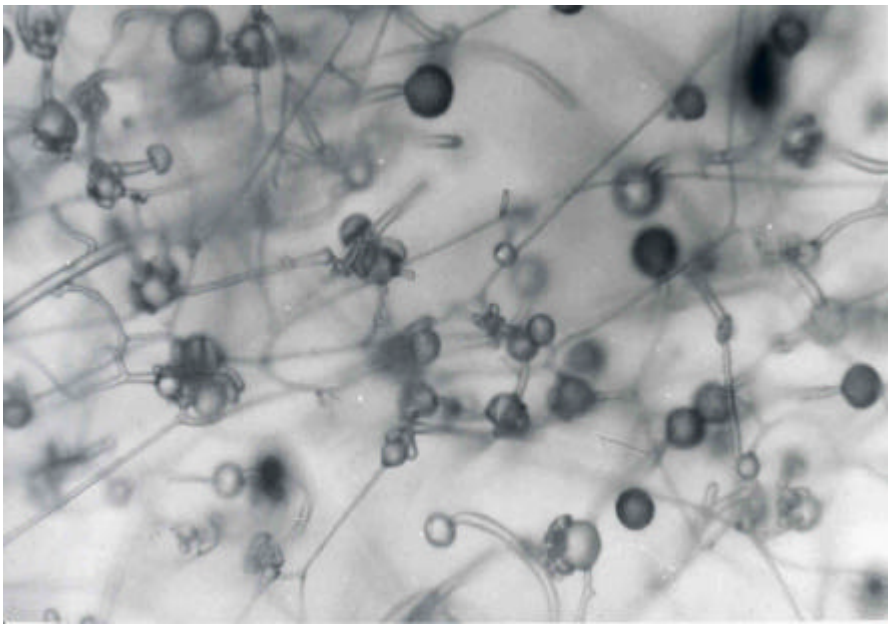


Fig 90: Parasitic interaction between *P. parasitica* (+) and *M. racemosus* (+)

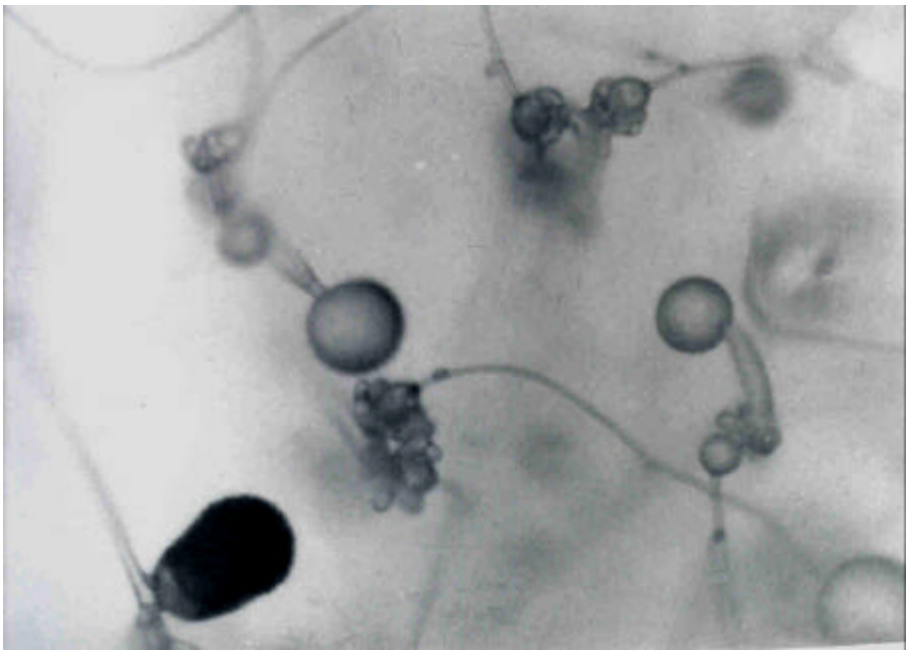


Fig 91: Parasitic interaction between *P. parasitica* (+) and *M. racemosus* (+)

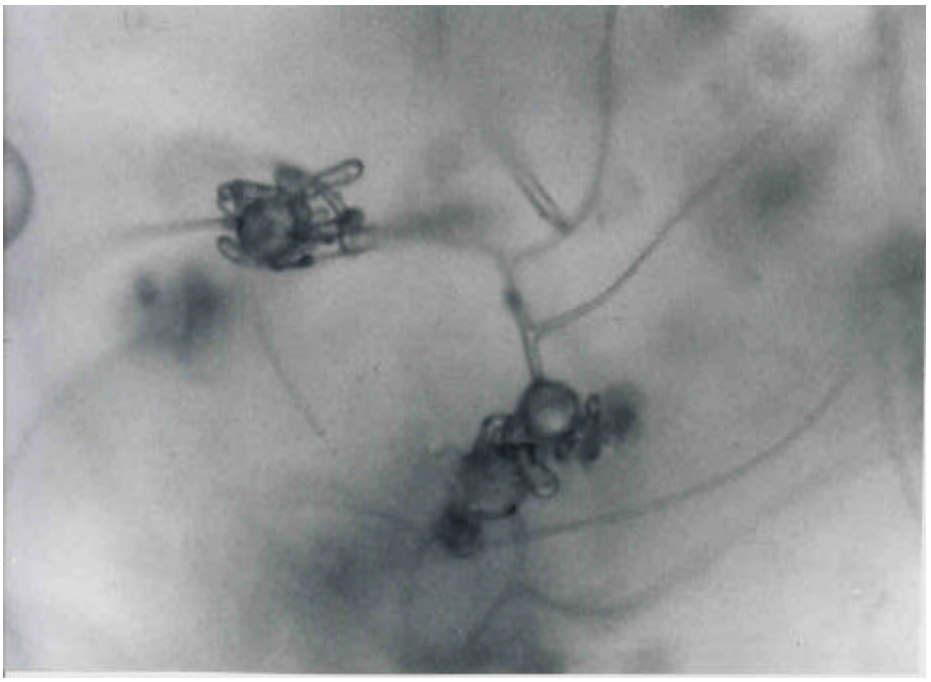


Fig 92: Parasitic interaction between *P. parasitica* (+) and *M. racemosus* (-)

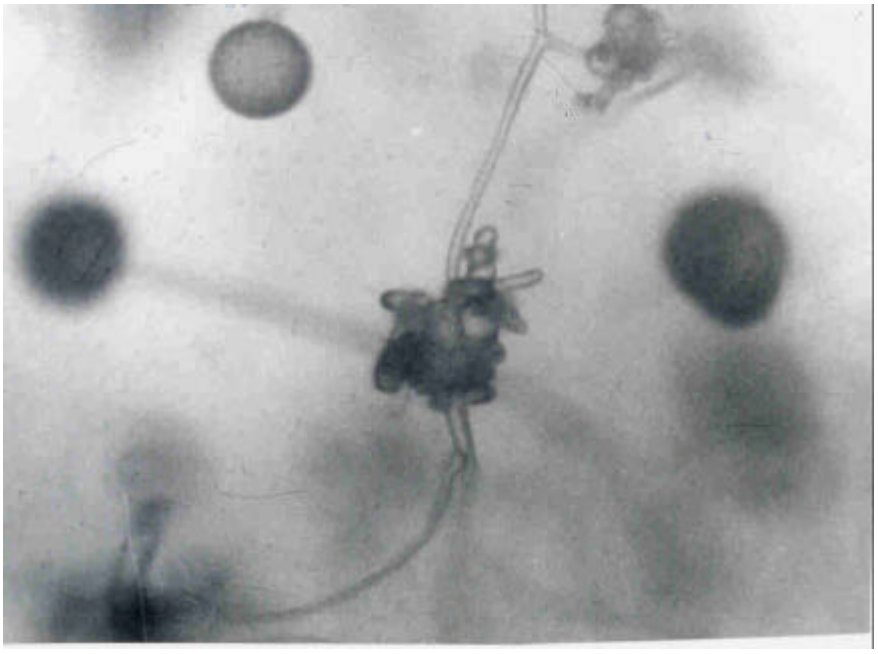


Fig 93: Parasitic interaction between *P. parasitica* (+) and *M. racemosus* (-)

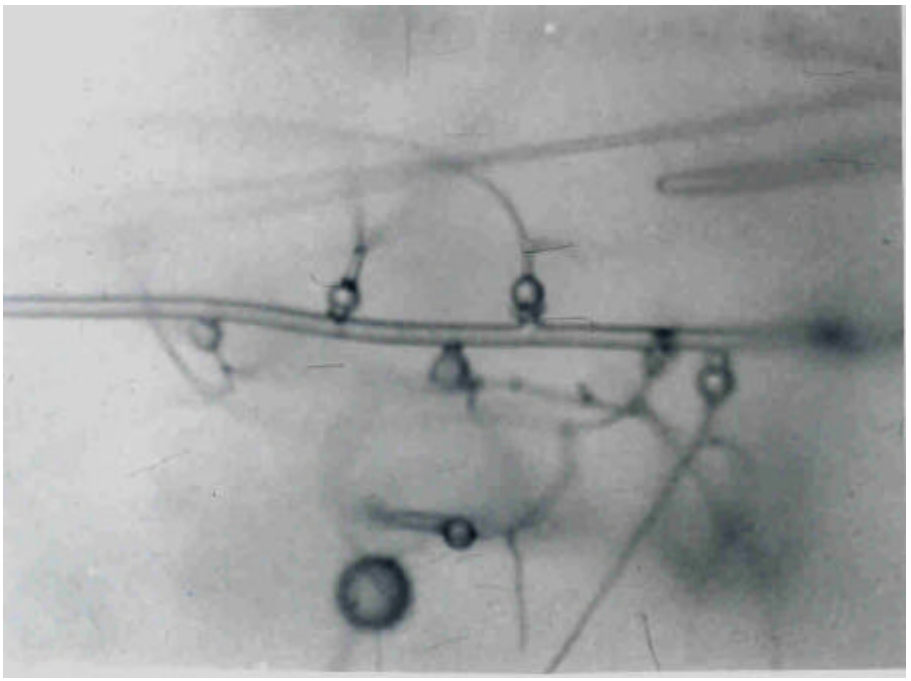


Fig 94: Parasitic interaction between *P. parasitica* (-) and *M. racemosus* (-)

Fig 95: Parasitic interaction between *P. parasitica* (-) and *M. racemosus* (-)

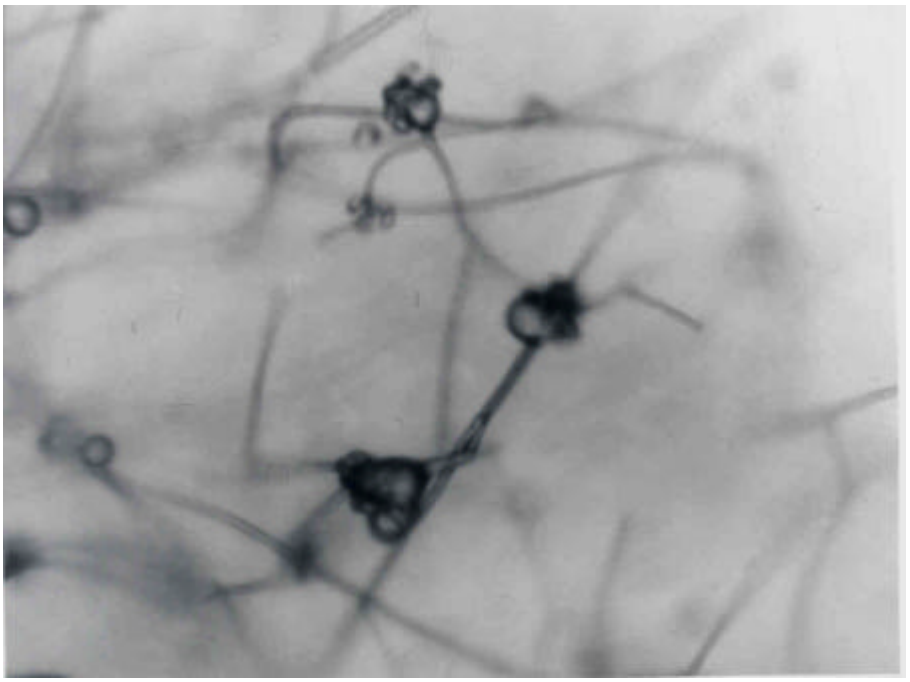


Fig 96: Parasitic interaction between *P. parasitica* (+) and *M. africana*

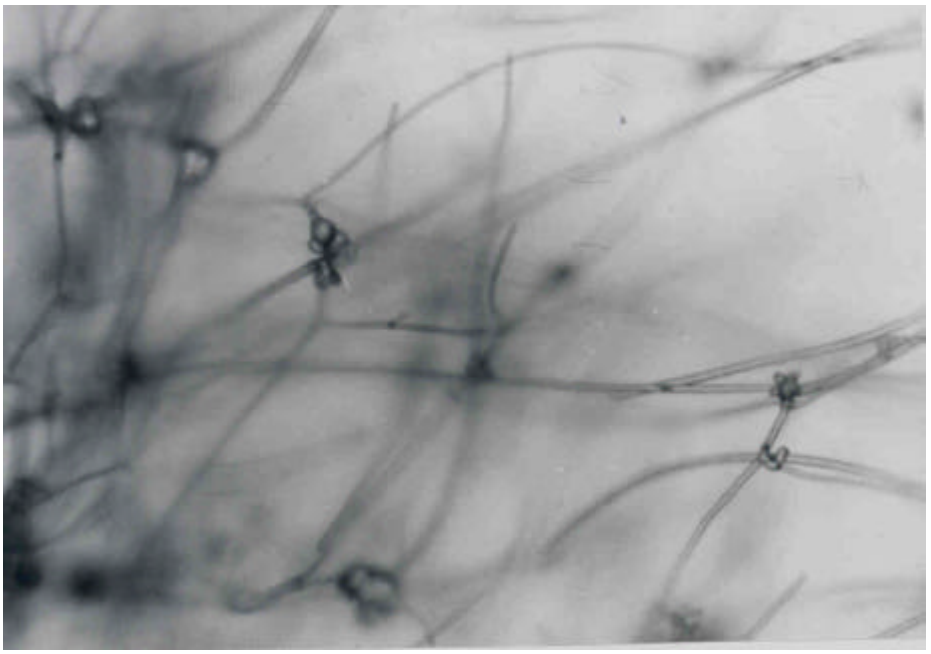


Fig 97: Parasitic interaction between *P. parasitica* (+) and *M. africana*

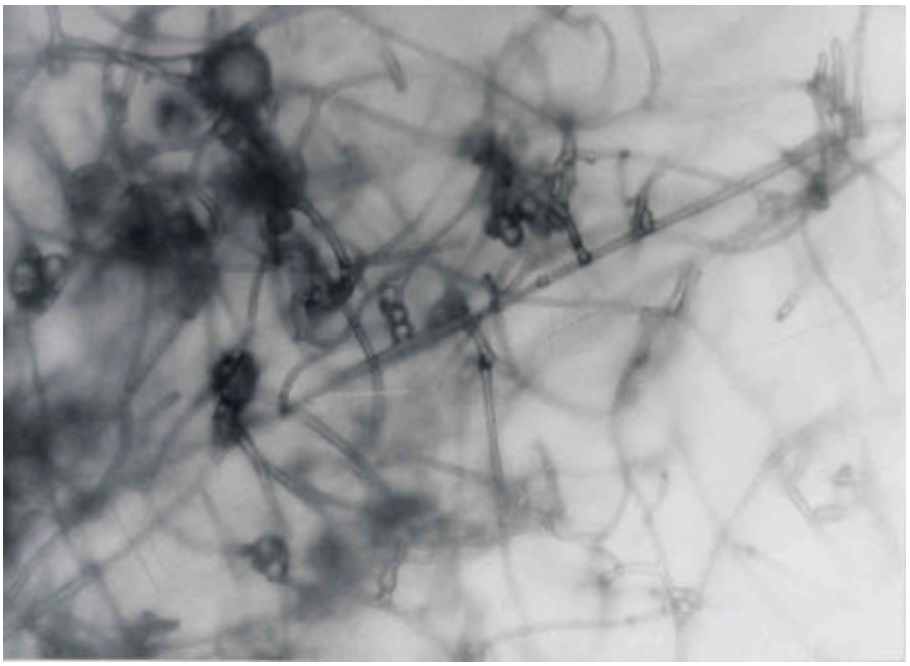


Fig 98: Parasitic interaction between *P. parasitica* (-) and *M. africana*

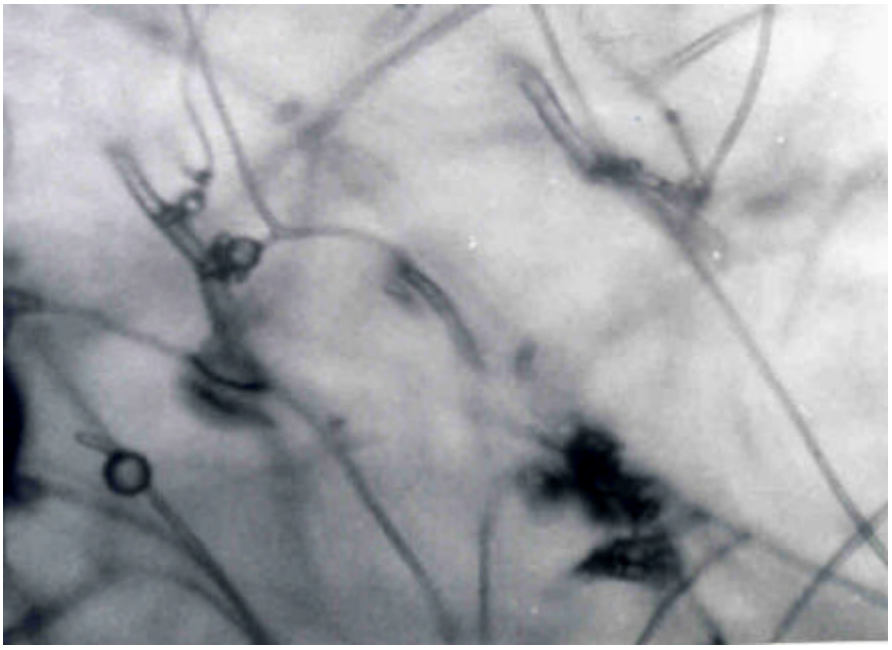


Fig 99: Parasitic interaction between *P. parasitica* (-) and *M. africana*

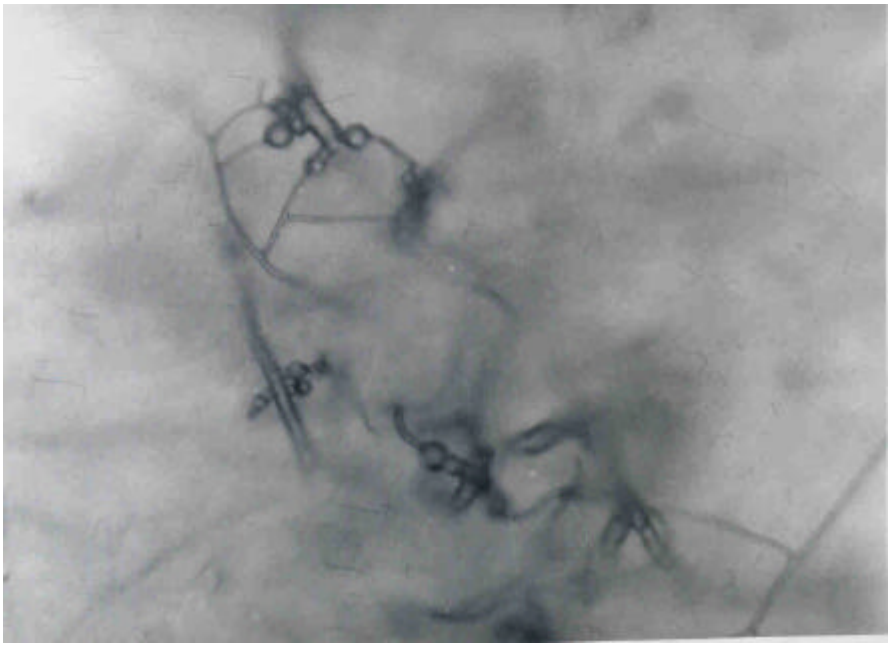


Fig 100: Parasitic interaction between *P. parasitica* (+) and *T. elegans* (+)

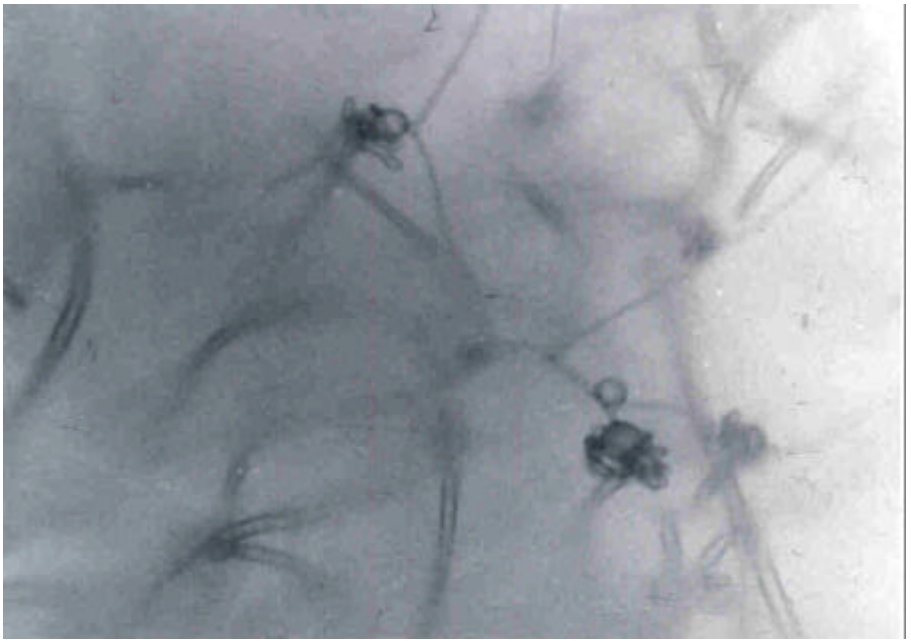


Fig 101: Parasitic interaction between *P. parasitica* (+) and *T. elegans* (-)

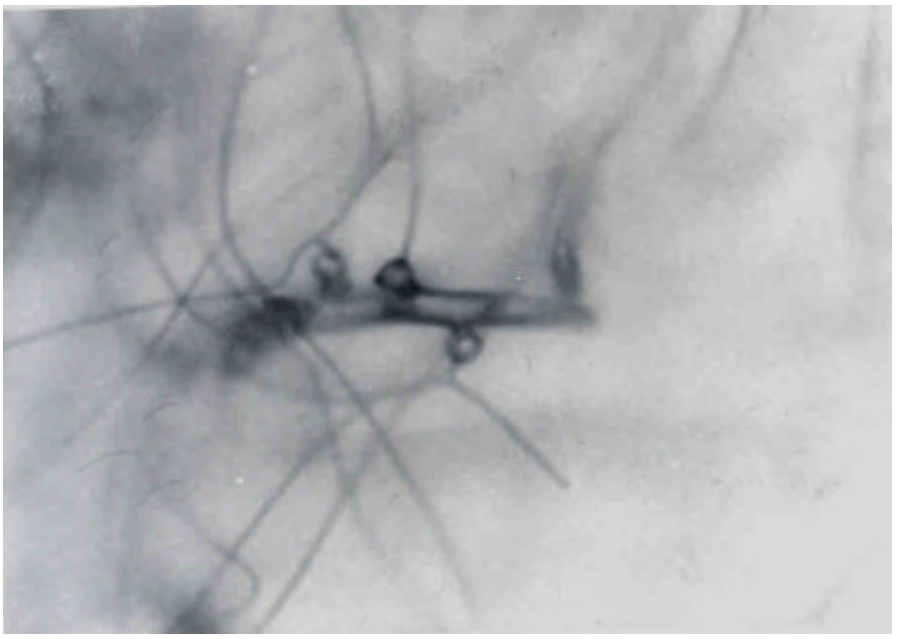


Fig 102: Parasitic interaction between *P. parasitica* (-) and *T. elegans* (+)

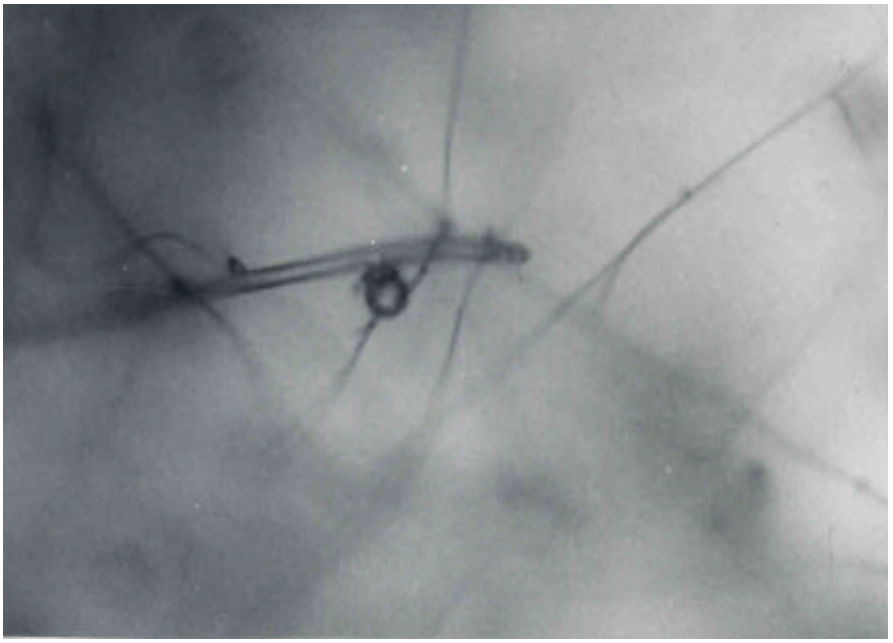


Fig 103: Parasitic interaction between *P. parasitica* (-) and *T. elegans* (-)

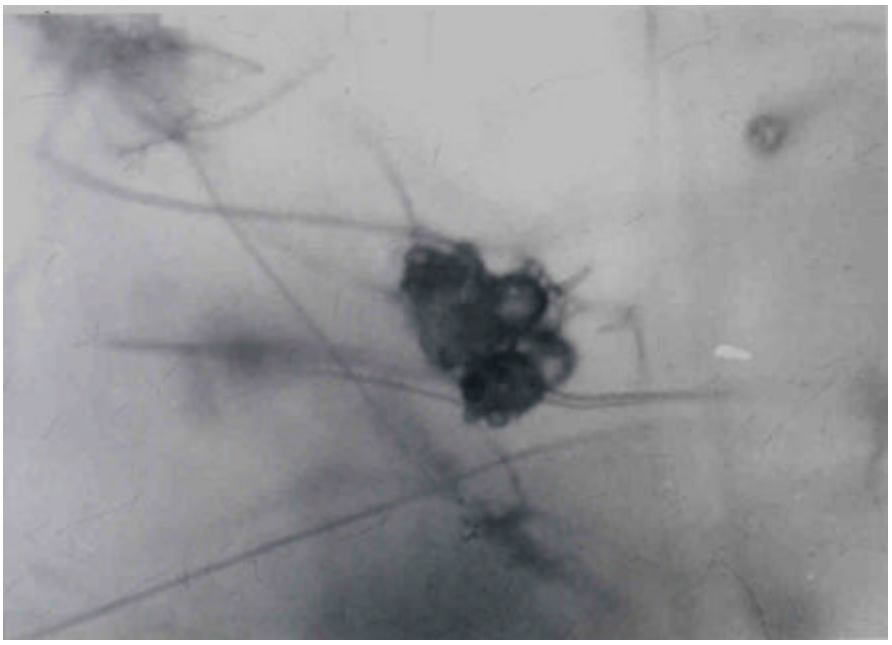


Fig 104: Parasitic interaction between *P. parasitica* (-) and *Z. moelleri*

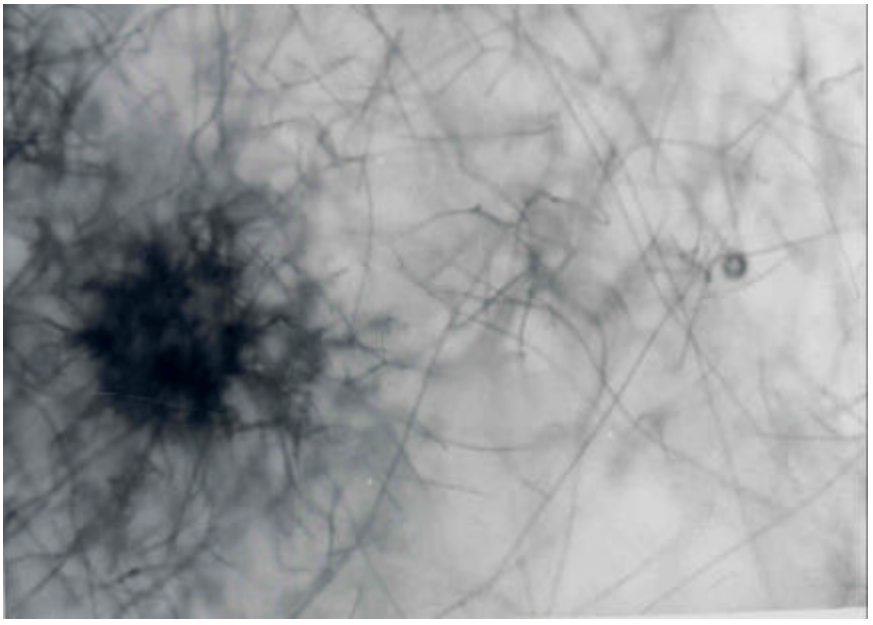


Fig 105: No parasitic interaction detected between *P. parasitica* (-) and *A. orchidis*

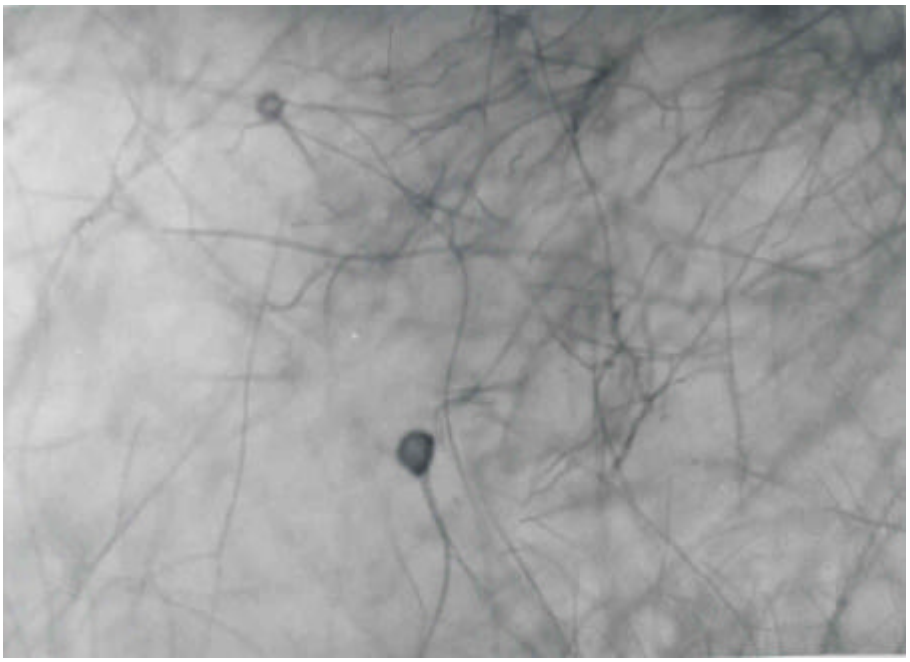


Fig 106: No parasitic interaction detected between *P. parasitica* (-) and *A. spinosa*

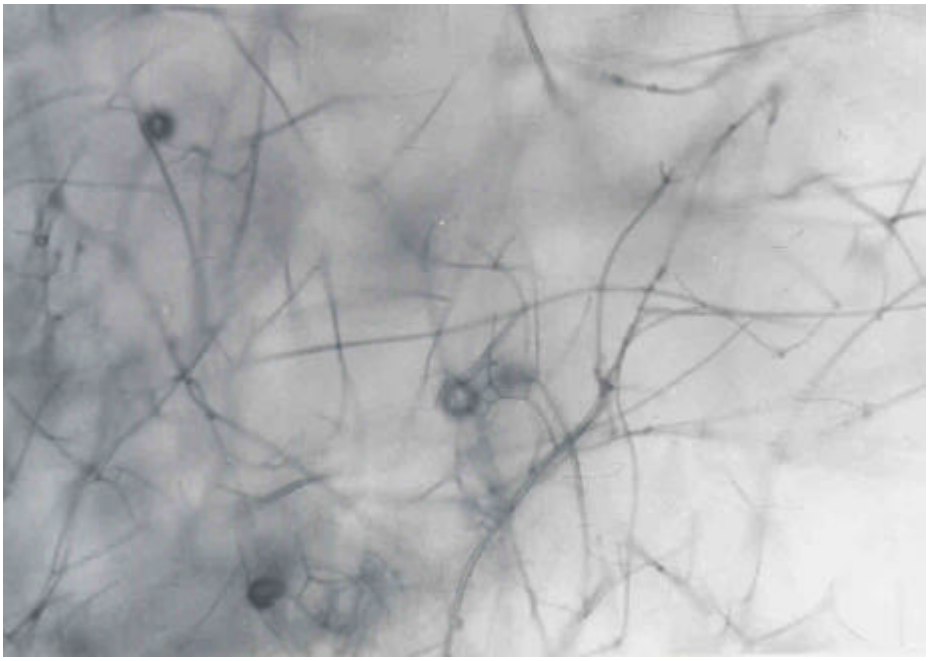


Fig 107: No parasitic interaction detected between *P. parasitica* (+) and *M. indohii* (-)

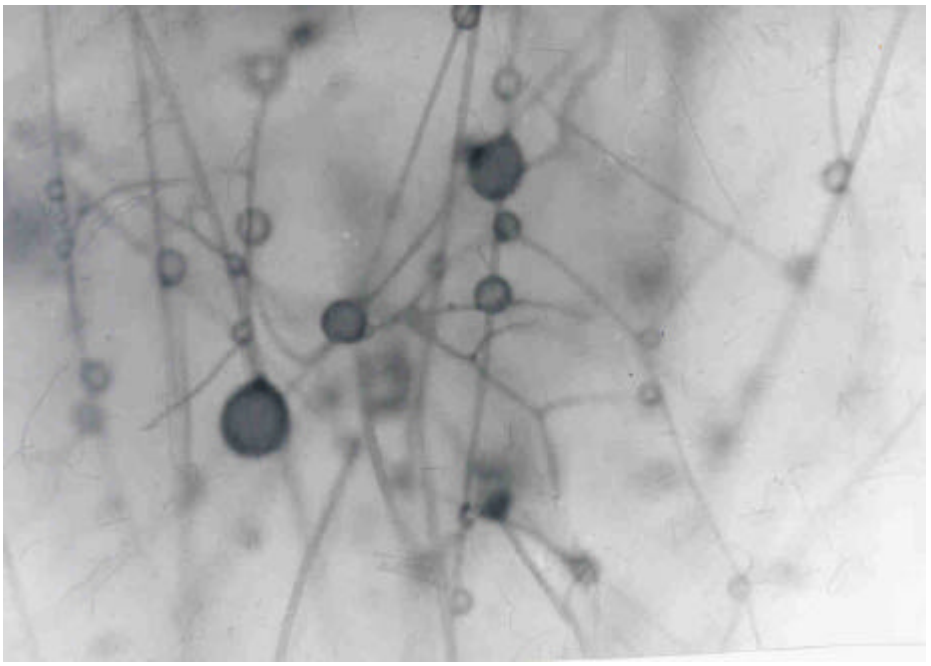


Fig 108: No parasitic interaction detected between *P. parasitica* (-) and *M. parvispora*

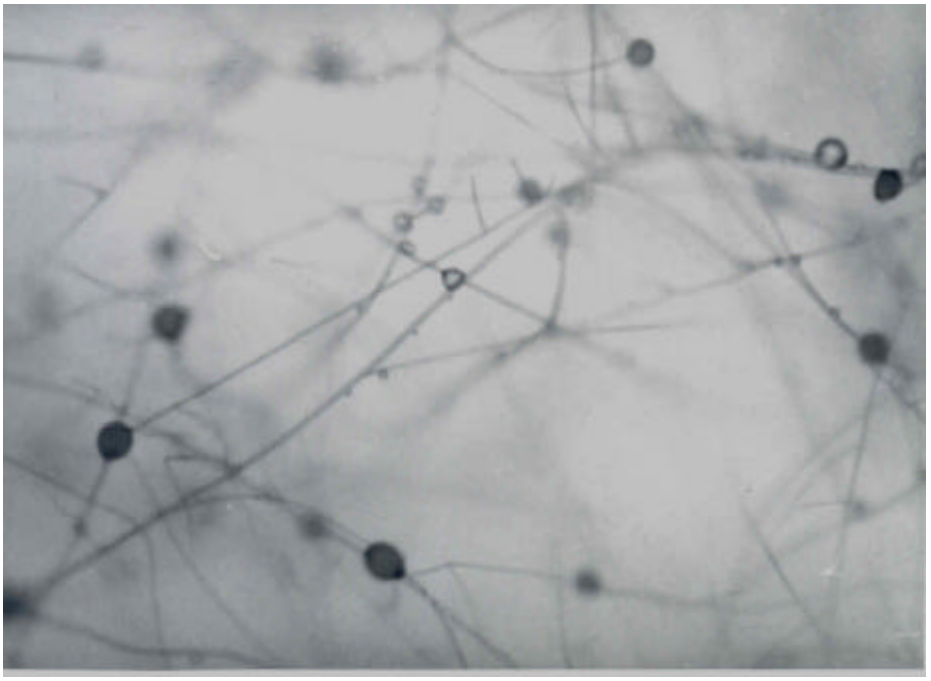


Fig 109: No parasitic interaction detected between *P. parasitica* (+) and *P. anomala*

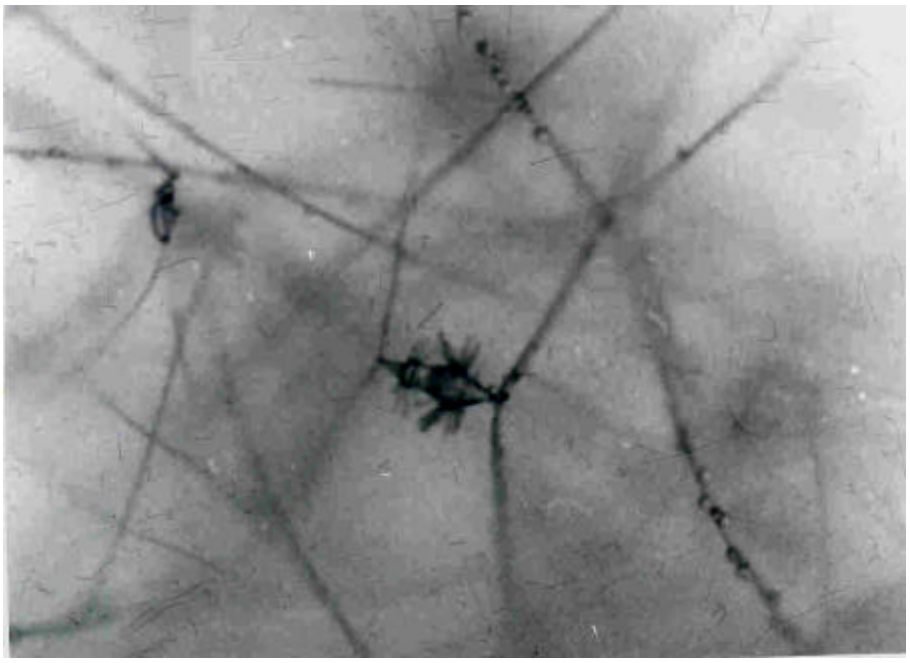


Fig 110: Sexual interaction between *A. glauca* 100.48 (+) and *A. glauca* 101.48 (-)

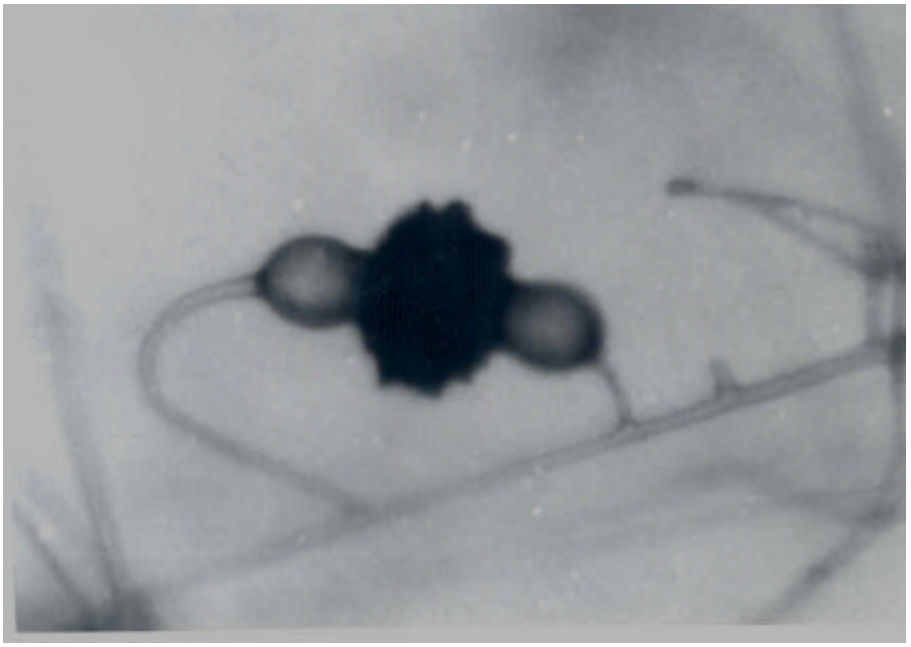


Fig 111: Zygospore formation in the homothallic *A. parricida*

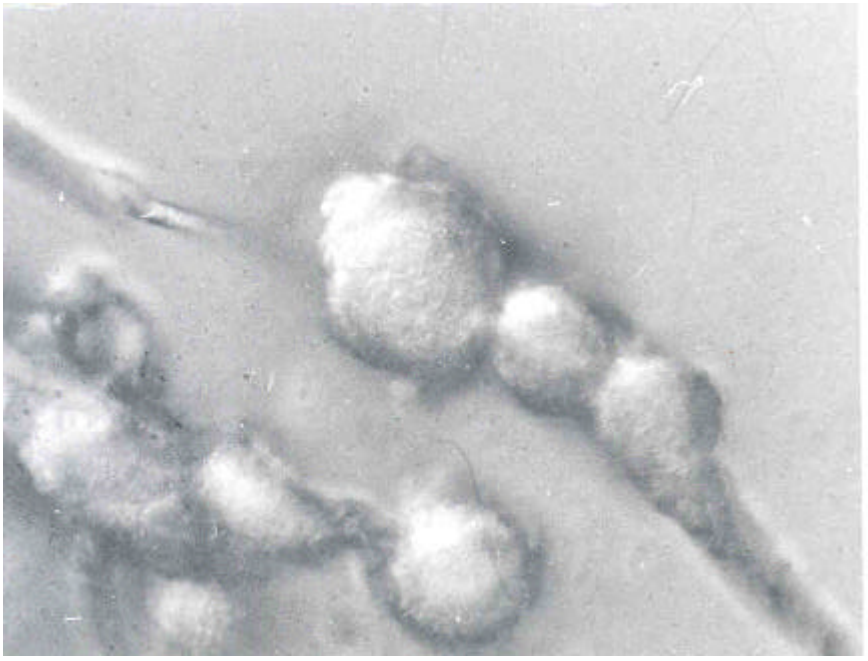


Fig 112: Zygospore formation in the heterothallic *M. indohii*

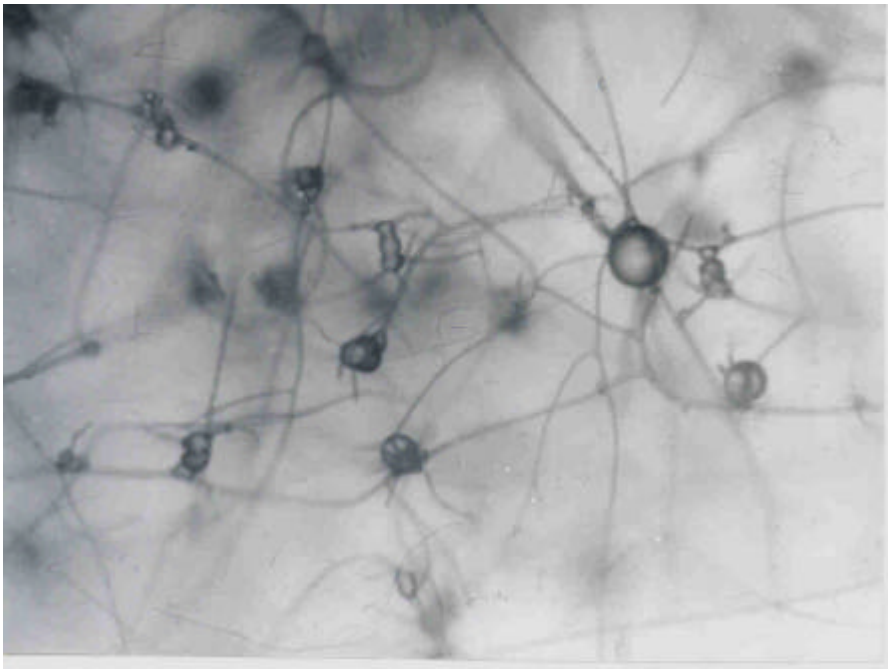


Fig 113: Sexual interaction between (+) and (-) mating types of *P. parasitica*

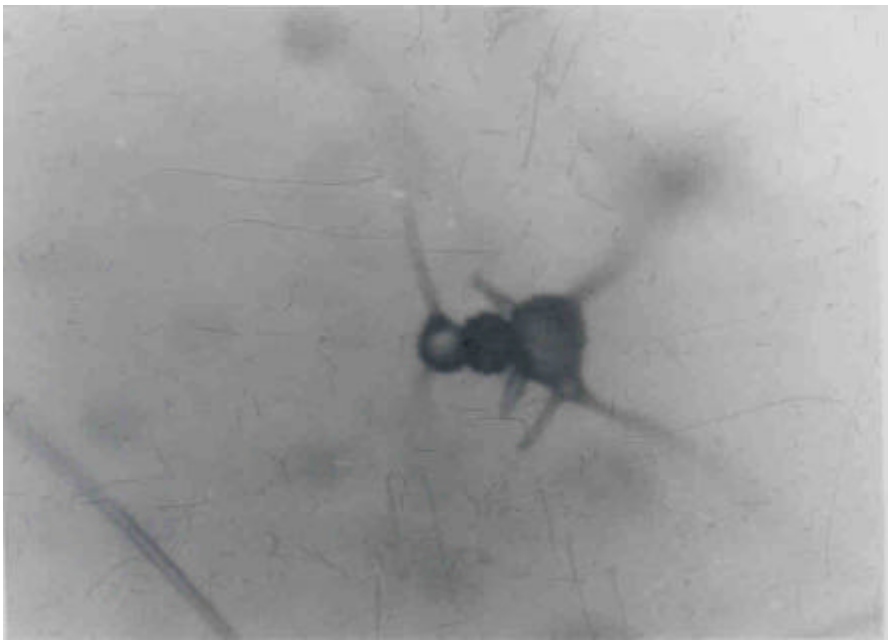


Fig 114: Zygospore formation in the heterothallic *P. parasitica*